FISH WELFARE

EDITED BY
EDWARD J. BRANSON

FISH VETERINARY SOCIETY

Blackwell Publishing

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Tribute

Edward Branson died suddenly before this book was finished and it is a great sadness to his friends and colleagues that he did not see the completion of a project that was so dear to his heart. Edward was a very active member of the Fish Veterinary Society and it was always his wish that the Society should address all aspects of fish welfare. It was Edward's ambition that the Society hold a wide-ranging workshop on current issues of fish welfare and, during his term of office as President, he saw this wish fulfilled – not without some difficulty. This book is a direct result of that workshop.

Edward obtained his veterinary degree from the Royal Veterinary College in 1979 and, after a variety of veterinary positions, decided to try fish medicine by enrolling

on an MSc course in aquatic veterinary studies at Stirling University. For the remaining 20 years of his life, Edward devoted himself to fish medicine and pathology and earned a reputation as a skilled diagnostician – he carried out pioneering work in several areas of fish disease, notably in salmon rickettsial syndrome and sleeping disease in trout. Disease prevention and fish welfare were always priorities with Edward and he was equally concerned over issues relating to the environmental impact and sustainability of the aquaculture industries.

Edward was a good friend for many years; he was a man of great intelligence, integrity and wit. It was a pleasure to have known him and a privilege to help in completing this book and seeing it published. Edward made an enormous contribution to fish health and welfare and this book is a very fitting tribute to a remarkable man and a dear friend.

Peter J. Southgate

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Blackwell Publishing would like to acknowledge the work of Edward Branson in compiling this important book, the manuscript for which we received just before Edward's most untimely death. Those of us who had worked with Edward to bring this project to completion would like to record our deepest sympathy to Edward's family.

We would also like to record our sincere thanks to Peter J. Southgate who most kindly stepped in to finalise and carry through editorial aspects of the book in Edward's stead.

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Introduction

Edward J. Branson and Peter J. Southgate

It is probably fair to say that in general, fish are considered to be inferior in evolutionary terms when compared to mammals and birds and, as a consequence, may be treated with less consideration for their welfare. However, as animal welfare is considered to be such an important topic in our society, for ethical and legal reasons and with an increasing interest in fish welfare and an accumulating body of scientific research on the subject, it is incumbent upon us to ensure that fish are not treated in such a way that their welfare may be compromised. Fundamentally: *is there sufficient evidence available to convince us that we need to bother with fish welfare and do we need to treat them with the same consideration and give them the same level of protection as mammals and birds?*

In an attempt to address this question, a meeting was held in Edinburgh in November 2004, organised by the UK Fish Veterinary Society (FVS), involving international representatives from a wide range of interest groups and including not only FVS members but also fish farmers, members of fish farming support companies, representatives of the Government and several non-governmental special interest groups. The purpose of the meeting was to attempt to review current knowledge relating to fish welfare and to determine if suffering can be judged. Clearly, if fish are considered to be sentient, capable of perceiving and consciously experiencing painful stimuli, then there would be a moral pressure to give them an appropriate level of protection, even if such protection is not enshrined in law.

Because there are many areas where human activity impinges on fish, not least angling and sea fisheries, an attempt was made to approach fish welfare from a general point of view and not solely with respect to fish which are under the care and control of man, that is, farmed and ornamental/exhibition fish. This book is a summary of the presentations given at the meeting, with extra material added in an attempt to give a fully rounded picture of the current state of knowledge.

Evidence was presented to show that fish have the same stress response and powers of nociception as mammals, and also that behavioural responses to a variety of situations suggest a considerable ability for higher level neural processing – a level of consciousness equivalent perhaps to that attributed to mammals. There certainly seems to be sufficient evidence to indicate an ability to suffer and, as a consequence, the overall feeling by the end of the meeting was that there was

indeed convincing evidence to show that fish should be treated with the same care and consideration, and therefore given the same level of protection, as mammals and birds. As a consequence, both the physical and psychological well-being of fish in our care need to be considered. It was clear that many valuable lessons can be learned about the assessment of fish welfare from mammalian and bird studies, but that it is equally important to remember that good welfare of fish cannot necessarily be achieved by a simple extrapolation from terrestrial animals and must take into account the complexities of fish behaviour and requirements which can, and do, vary both between and within species. In summary, although research into fish welfare was seen to be progressing well, it was also clear that there are still large areas where little is known and research is necessary before we can have a fuller understanding of what actually constitutes good welfare for all species of fish in our care.

All speakers at the meeting were UK-based, but the information given reflected the state of international knowledge and research. In addition, the social approach to fish welfare in different countries was also considered, with possibly the most interesting being Germany where fish welfare is taken very seriously. If it is established that fish need to be treated with the same care and consideration as birds and mammals, questions may be raised with respect to the use to which we put fish in our society.

This must include aquaculture, the keeping of ornamental fish, capture fisheries and angling. For example, will our approach to angling need to be reassessed? Certainly in contrast to many other European countries, Germany already affords a very high degree of protection to fish; here, animal welfare, including that of fish, is considered to be a constitutional goal and if welfare competes with another issue, as a constitutional goal, welfare will have priority. Amongst other things, this has the effect of making put-and-take fisheries, catch and release, live bait, angling competitions and keep nets illegal, and anglers must pass basic tests of competence before being allowed to practise their pursuits. In the UK there are currently no controls on who becomes an angler, or on whether they need to have any knowledge of fish handling, welfare and the possible pain and suffering which can be afflicted upon the fish during this process. This situation will not change under the recent Animal Health and Welfare Act.

Concern was raised in 1996 by the Farm Animal Welfare Council in its Report on the Welfare of Farmed Fish, particularly in the areas of stocking density and slaughter.

There has been a substantial body of active research into many aspects of fish welfare and, while many improvements have clearly been made since the report, some concerns still exist and a few areas where continuing research is necessary were identified.

The question of welfare on fish farms was addressed during the presentations and it became clear that, although some parameters which may have an impact on fish welfare are measurable, e.g. stocking density and water quality, the actual

assessment of on-farm welfare was subject to a great deal of uncertainty. An objective of a Defra/British Trout Association funded project on fish welfare is to produce a standardised protocol for assessing fish welfare. After hearing the speakers, attendees at the meeting were asked to consider which key parameters, in their opinion, should be included in such an assessment. The deliberations of the groups are summarised here.

Each chapter of this book has been written by specialists in their field, frequently containing more material than was presented at the Welfare workshop. The subject matter is wide-ranging and covers in detail, concepts of animal welfare in addition to more specific aspects of fish welfare. Philosophical concepts of welfare are discussed along with more practical areas of fish welfare encompassing all husbandry and management activities that have a potential to affect the welfare of the fish in our care.

The Fish Veterinary Society (FVS) is a specialist division of the British Veterinary Association that aims to bring together fish veterinarians and other fish health professionals to advance the veterinary care and welfare of fish. The Society acknowledges the time and effort put in by all the speakers and the help and assistance given by members of the FVS Committee, in particular Fiona Macdonald and Chris Walster.

Part I

General Fish Welfare

Chapter 1
What is Animal Welfare?

Alistair B. Lawrence

Introduction

Animal welfare in one sense is a commonly observed concern of humans to situations where animals appear to be suffering. Media coverage of diverse events such as the beaching of whales, participation in 'blood sports' and acts of direct cruelty to animals reflect this public interest in animal welfare. The Fish Veterinary Society (FVS) meeting, Fish Welfare, itself indicates that public concern for animal welfare which originally focused on mammals and birds, is now increasingly directed at other species including fish (Chandroo *et al.* 2004), and some invertebrates (Sherwin 2001).

In answering the question: 'What is animal welfare?' the development of animal welfare as a societal issue will be described first, focusing mainly on the UK. This historical perspective will provide a basis for evaluating the different explanations of why humans express concern for animal welfare. For example, we can ask whether the historical evidence supports the idea that animal welfare is a recently emerged phenomenon resulting from our greater wealth and hence freedom to consider issues that are not directly linked to our own survival. How science can be applied to address animal welfare issues of public concern will then be considered.

Animal welfare: the long view

Some of the most direct historical evidence of human attitudes to animals comes from the various ways that religion has dealt with animals, and human treatment of animals.

Animals and religion have been intertwined from the earliest records, with depictions of animal deities being found with the remains of prehistoric human society (Sax 1994). However, from these early beginnings, 'modern' religions have developed an almost bewildering variation in the moral status they allow animals. A conventional view of this complexity suggests that some religions, such as Jainism and Buddhism, allow animals a higher moral status than other religions (e.g.

Christianity, Judaism) which tend to see animals as being under human dominion and as lesser creatures (Maybury-Lewis 1992 cited in Preece & Fraser 2000, Raj 2004).

One interpretation of the variation in the moral status that religions allow animals, is that religions are influenced by different views on whether animals are sentient, where sentience refers to the capacity of animals to experience or 'feel' in a way that is analogous to human experience. Thus religions that give animals a higher moral status such Hinduism also see animals as sentient (Raj 2004). Christianity is interesting in this respect. Christian doctrine sees mental states as important in determining the moral status of a subject (Cohen & Rozin 2001). Yet, Christianity has been influenced by a diverse set of views on the mental states (sentience) of animals (Preece & Fraser 2000). This variety of views on animal sentience could explain in part the array of different views that Christianity has regarding animals' moral status and the existence of animal souls (Waldau 2000).

The relationship between religious beliefs and attitudes, and actual behaviour to animals is similarly complex. For example, those religions that treat animals as soulless, such as Islam, also tend to promote compassion to animals (Alboga 2003). Furthermore, whilst religion is often seen as a factor influencing attitudes to animal welfare issues when assessed by survey (Hagelin *et al.* 2003), religion apparently does not influence the level of distress experienced on the death of a pet (Davis *et al.* 2003). Attitudes and behaviour on kibbutzim in Israel found that there was no less concern and action for dairy cow welfare on non-religious as opposed to religious kibbutzim (Rabbie 2000).

Moral philosophy involves the development of systematic approaches to determining 'right' or 'wrong' behaviour (Rawls 2000) and has had a major influence on the development of attitudes to animals. There has been a great diversity of philosophical writings regarding the moral status of animals. A significant part of this debate relates to the question of animals' mental state or degree of sentience. On this issue, philosophers have spanned all possible views from those of Descartes, who saw animals as effectively inanimate, to others who regard animals as having the capacity to experience or 'feel' in a way that is qualitatively similar to humans. For example, the Scottish philosopher David Hume wrote in 1742 that: 'animals undoubtedly feel ... tho' in a more imperfect manner than men' (Hume 1987 (revised edition)). Perhaps the most famous early advocate for animals' moral status was Jeremy Bentham (1789) who suggested that: 'the question is not, can they reason nor can they talk but can they suffer'.

Early philosophers such as Hume and Bentham were influenced by emerging scientific evidence of the biological similarities between human and non-human animals which called into question the anthropocentric view that dominated at the time (Radford 2001). Scientific investigation of animals from the eighteenth century has continued to illustrate the extent of animals' mental capacities and can be argued to provide scientific support for non-human animal sentience (Griffin 1992, Dawkins 1998). It is also important to acknowledge the important limitations

of the scientific approach when applied to the issue of animal sentience (see next section).

The ensuing development of a more positive attitude to the moral status of animals was responsible for the first early steps to protect animals in law. As described by Radford (2001), the passing of early animal protection legislation coincided with the writings of Bentham and others and the beginnings of a changing attitude to animals' moral status. Initially, legislation was focused on preventing direct acts of cruelty against animals (e.g. Martin's Act, 1822, to protect animals in London's markets of the day). The emphasis has been subsequently replaced by legislation aimed at protecting animal welfare in a broader sense. Animal protection legislation has increasingly gone beyond defining minimum standards, to defining how animals ought to be cared for, a process that continues to the present day in the form of the Animal Welfare Act (HMSO 2006), where the concept of a duty of care is extended beyond farm animals to all animal uses. Arguably one of the most significant aspects of current animal welfare legislation in the UK and Europe has been the acceptance in law that animals (or at least vertebrates) are sentient. For example, paragraph 10 of the Explanatory Notes to the Animal Welfare Bill reads that: 'The Act will apply only to vertebrate animals, as these are currently the only demonstrably sentient animals' (House of Commons 2005). However, Clause 1(3) makes provision for the appropriate national authority to extend the Act to cover invertebrates in the future if they are satisfied on the basis of scientific evidence that these too are capable of experiencing pain or suffering.

In the UK, the recent publication of the GB Animal Health & Welfare Strategy (AH&WS) in 2004 marks a critical step in the development of farm animal welfare policy (Defra 2004). The Health and Welfare Strategy builds on the historical basis of farm animal welfare concerns. It uses the 'five freedoms' (see FAWC 2005) to emphasise the wide scope of welfare issues affecting animals and the importance of both animals' physical and mental well-being. However, it also highlights a policy shift from an almost complete reliance on regulation as the most effective mechanism to improve welfare in favour of non-legal approaches such as partnerships, shared responsibilities, prevention and an understanding of the costs and benefits of welfare improvements. The implementation of the strategy (e.g. SEERAD 2003) is increasingly aimed at encouraging a more proactive stance on health and welfare by the farming industry, and partnership with related support services such as local veterinary practices. There is now a distinctive trend towards countries developing similar health and welfare strategies, which place less emphasis on legislation as the main route to improving welfare. For example, the recently published EU Animal Welfare Action Plan aims to improve animal welfare over the next five years by promoting research and alternative approaches to animal testing, and introducing standardised animal welfare indicators (EU 2006).

In summary, the history of the debate over the moral status of animals informs us that this is a long-standing concern which stretches back many centuries pre-dating the modern era. It is not possible to argue therefore that concern for animal welfare

is simply a reflection of our current affluence and ready supplies of cheap food. It is also clear that concern for animal welfare is not necessarily a direct reflection of religiously motivated debate. In the UK, animal welfare concerns are more closely associated with the scientific and rational perspective of the relationships between humans and other animals that has developed since the eighteenth century. One important result of scientific research into animal behaviour and welfare has been to provide evidence of animals' motivations, their mental capacities and by inference, support for animal sentience. This scientific support for animal sentience has provided the basis for EU and UK legislation that enshrines the concept of animal sentience in law. Scientific evidence relating to animal sentience may continue to be critical to further advancing the moral status of animals. Socio-economics research suggests that the moral characteristics we associate with an animal welfare issue are important in determining the moral importance we place on the issue and our consumer behaviour (Bennett *et al.* 2002). Recent work has also shown that belief in animal mind is an important predictor of attitudes to animals (Knight *et al.* 2004). For the future, this suggests that further scientific support for animal sentience should increase the moral importance of animal welfare through widening societal acceptance and belief in the animal mind. In some ways it is hard to see a limit to this process. Somewhat tongue in cheek, a recent *New Scientist* article foresaw a time when the moral complexities of our lives with animals had become so complex that it had led to humans living separate lives from animals that were then left to their own devices (*New Scientist* 2005). Coming back to the topic of the FVS meeting, fish welfare provides an excellent example of how interest in the welfare of fish has led to an increase in scientific activity in the area, as demonstrated by the contents of this publication. This research has in turn, helped lead to an increasing profile for fish welfare over a relatively short period of time and calls for their greater protection in law. On the basis of the recent past we might therefore expect in the future to see even greater pressure applied to the conditions applied to farmed fish, and to other welfare concerns associated with angling and the harvesting of wild fish.

Science applied to animal welfare

Science has long played a critical role in the development of concerns for animal welfare. As we saw earlier, the writings of philosophers such as Bentham and Hume coincided with a growth in scientific observations pointing to the many similarities between animal and human form and function (Rigotti 1986). The Brambell Committee which sat following the publication of *Animal Machines* (Harrison 1964) was strongly influenced by the science of the day including the growing understanding of animal psychology and behaviour (Brambell 1965). As already discussed, the developing legal status of vertebrates as sentient beings owes much to discoveries relating to the cognitive and emotional capacities of animals.

For animal welfare where there can be diverse opinions over sore issues such as the nature or even existence of animal sentience, science can potentially provide a rational evidence base from which to develop policy and interventions. However, inevitably there are important disagreements over the scientific approaches applied to animal welfare. As Keeling (2004) recently wrote: 'scientists ... tend to disagree on interpretation rather than emphasising similarities'. Fraser *et al.* (1997) attempted to produce a more consensual approach by identifying three main scientific philosophies, which have been used to address animal welfare issues. These are in summary that: (a) animals should live natural lives; (b) animals should feel well; (c) animals should function well. In the past, advocates of these different philosophies have disagreed over their relative importance. For example, McGlone (1993, cited in Fraser *et al.* 1997) wrote in support of the 'functioning approach' that: 'I suggest that an animal is in a poor state of welfare only when its physiological systems are disturbed to the point that survival or reproduction are impaired'. In contrast, Duncan (1993, cited in Fraser *et al.* 1997) wrote in support of the 'feelings approach' that: 'welfare is dependent on what animals feel'.

An exemplar can be used to demonstrate that these different approaches can provide complementary rather than opposing interpretations of welfare in a given situation. For this it is necessary to use an issue which has received a significant amount of scientific attention, and where there is sufficient data both to describe the use of these diverse approaches and to assess the interpretations that emerge when animal welfare is viewed from different scientific perspectives. One such issue is the widespread use of crates to house the parturient (farrowing) sow in order to protect piglets and to ease management of the sow and her newborn litter. These crates are also behaviourally confining and prevent the sow from turning her body. The scientific evidence on the impact of this close confinement on the sow can be arranged into the three scientific approaches identified by Fraser *et al.* (1997).

Living natural lives

The philosophy underlying this approach is that animals' evolved adaptations will often be retained even in today's domesticated strains and that these adaptations can come into conflict with the current conditions in which we keep animals. Evidence to explore this hypothesis comes from studies of the wild type of the species and from the domesticated strain under 'natural' or 'confined' conditions.

Wild boar sows are described as isolating themselves from the social group as parturition approaches, selecting a nest site and then constructing a nest from materials such as branches and grasses which they gather and carry from surrounding areas (Nowak 1991). Near the onset of parturition, the sow enters the nest to deliver her piglets, tending not to emerge until 4–7 days post-birth. Similar behaviour has been described in domestic sows under free-ranging conditions including sows which have been reared under intensive farming conditions (Stolba & Wood-gush 1984, Jensen 1989). There is evidence that the nest-building behaviour that

precedes parturition is triggered by release of prostaglandins as part of the essential endocrine cascade associated with parturition in the pig (Gilbert *et al.* 2001). Sows which are housed in closely-confining parturition crates are often described as displaying 'restless' behaviour over approximately the same time period that nesting behaviour is expressed in free-ranging sows. Detailed behavioural analysis shows that pre-parturient crate-housed sows increase their activity levels and their pawing and nosing of the crate floor and fittings (Jarvis *et al.* 1997).

Taken together, these data suggest that parturient nest building in the sow is an adaptation that ensures better survival of the neonate piglet through protection against cold and predation and is triggered by specific hormonal changes that accompany the onset of parturition (Jarvis *et al.* 1999). Sows in crates are similarly stimulated by their physiology to express nest building but the crate so strongly restricts the behaviour that it is barely recognisable and is effectively a 'redundant' response in the crate environment (Fraser *et al.* 1997). This analysis raises the question of how the potential conflict between an animal's biology (arrived at through the processes of natural selection) and the artificial conditions in which it is kept as a domestic animal might be reflected in its emotional and functional responses.

Feeling well

The need to consider the mental state (or 'feelings') of animals was clearly emphasised by the Brambell Committee (Brambell 1965), but has remained one of the most controversial aspects of the application of science to animal welfare. The range of views is extreme. Some scientists believe that this area cannot be addressed scientifically (Kennedy 1992), whilst others (Duncan 2005) suggest that animal feelings is the core issue that must be addressed if we are to fully incorporate the animals' perspective into our assessments of animal welfare issues. The scientific investigation of animal feelings is clearly linked to our understanding of animal sentience, which as we have seen has become increasingly important in determining public attitudes to animal welfare.

A number of methods have been used to assess animal feelings in the context of animal welfare. All of these approaches have implicit or explicit assumptions, reflecting the intrinsic problem of measuring subjective (private) experiences in non-verbal animals. One approach is to allow animals to express their motivational preferences or choices and to assume that these expressed preferences reflect the animal's subjective feelings (Hughes & Black 1973, Dawkins 1980). Development of preference testing has led to the concept that animals can express their motivational priorities (or 'consumer demand') if they are required to 'work' for access to resources (Mason *et al.* 2001). Arey (1992) used the consumer demand approach to assess the motivational priorities of sows in the pre-parturient period (Arey 1992). He showed that given the opportunity to work for access to food or straw, sows' motivation for straw (presumably to nest build) approached equivalence to that for

access to food in the 24 hours preceding the onset of parturition. The assumptions underlying the consumer demand approach imply that sows' feelings will reflect their motivational state, with sows experiencing positive feelings when able to nest build during the nest-building phase, and correspondingly negative feelings when denied the opportunity to nest build.

Functioning well

The concept that animal welfare can be assessed through effects on biological functions is primarily based on the extensive literature of the effects of animal and human 'stress'. Since the early work of Weiss and others (e.g. Weiss 1972) began to demonstrate the impact of psychological stress on disease states, studies have shown the wide-ranging effects of stress on growth, reproduction, the immune system and behaviour. In the context of animal welfare, research has often focused on the physiological pathways involved in mediating stress effects as a means of indicating stress and by inference, poor welfare. The most widely studied of these pathways has been the hypothalamic–pituitary–adrenal (HPA) axis which in vertebrates is highly sensitive to a range of environmental 'stressors'.

There is good evidence in the sow that confinement in crates during the nest-building phase is 'stressful' given its effects on the HPA axis. For example, parturient sows in crates show increased adrenocorticotropic hormone (ACTH) and cortisol concentrations during the nesting phase relative to controls able to nest build in open pens (Jarvis *et al.* 1997), a difference which disappears once the nesting phase is over and parturition starts (Jarvis *et al.* 2004). It is also clear through the comparison of ACTH and cortisol concentrations across treatments that the activation of the HPA axis in crated gilts is not solely a response to the onset of parturition (Jarvis *et al.* 1997).

There is debate over the wider significance to welfare of increased HPA activation. It is not always clear where the threshold lies with respect to 'normal' adjustments of physiological systems such as the HPA axis, and more significant perturbations that may indicate an increased risk to mental or physical health (Mendl 1991). One way of resolving this is to demonstrate that a stressor has not only affected systems such as the HPA axis, but that there are also significant effects on biological functioning. In the case of the parturition crate, evidence suggests that abnormal maternal behaviour, in the form of biting at and direct physical attacks ('savaging') by the sow on her piglets, is triggered by the crate environment as savaging is much more common in that environment (Jarvis *et al.* 2004). Jarvis *et al.* (2006) found that female offspring of sows that were socially stressed during pregnancy developed characteristics of stress hypersensitivity in terms of their brain development, and physiological response to stressors. These prenatally stressed offspring showed a tendency to bite at and attack their piglets when housed in farrowing crates, suggesting that the crate environment was sufficiently stressful to trigger abnormal maternal behaviour in these stress sensitive animals.

Taken together, this evidence provides a coherent picture of the welfare implications of parturition crates and at the same time generally illustrates the application of different scientific approaches to a welfare issue. The animal's evolutionary background can be important in dictating its response to even highly intensive farming conditions. In the case of the domestic sow, the motivation to nest build has evolved because of its advantages to piglet survival. The physiological basis of the behaviour makes it an unavoidable aspect of the parturition process even when sows are housed in behaviourally-confining crates. Animal feelings, although a controversial subject, can be approached scientifically given certain assumptions. For example, 'preference testing' and 'consumer demand' approaches assume a close correspondence between animal motivations and feelings. Studies in the sow have shown that there is an increasing motivational priority to nest build in the 24 hours that precede parturition. We can assume that the sow experiences positive emotions when allowed to express her nesting motivation and negative emotions when the motivation is thwarted as in the parturition crate. We might predict that these emotional responses would be reflected in terms of their wider impacts on biological functioning for example through activation of 'stress' sensitive pathways such as the HPA axis and on functional outcomes such as health, production and behaviour. Indeed, in the case of the parturient sow, the thwarting of nesting behaviour by the crate (and the assumed negative emotional state that results) is associated with increased indications of physiological stress. Furthermore, there is evidence that this stress may be involved in triggering abnormal maternal behaviour in the form of physical attacks by the sow on her piglets. Similarly in mink, which have evolved a partially aquatic lifestyle, research has demonstrated a high motivational priority for access to water, and that where that motivation is thwarted (as it often is when mink are farmed for fur), there are clear indications of physiological stress (Mason *et al.* 2001).

These studies show a clear correspondence between the different approaches used in applying science to welfare issues, demonstrating that some farming methods can interfere with evolved (motivated) behaviours causing significant perturbations to physiological systems, and to biological functioning. It is often assumed in welfare studies that important mediators of such effects are the animals' feelings. In the example of the parturient sow, we might assume that the thwarting of nest building by the crate induces a state of 'frustration' and that it is the (mental) state of frustration which underlies the (physical) functional responses (e.g. HPA activation, abnormal maternal behaviour).

It has to be acknowledged that other welfare issues have proved more difficult to interpret given the lack of correspondence between the different scientific approaches used in their assessment. For example, there has been much concern over the welfare effects of food-restricting breeding populations of pigs and poultry (broiler breeders). In broiler breeders a number of studies have failed to find a consistent set of relationships between behaviour and physical and physiological

responses to food restriction (e.g. de Jong *et al.* 2003). One reason for this may be that we have yet to fully understand the evolutionary ‘rules’ that animals use to govern their food intake which makes it difficult to interpret when animals might be experiencing different levels of hunger (Lawrence *et al.* 2004). These more intransigent problems indicate the continuing need to develop the theoretical and philosophical basis upon which we base the application of science to animal welfare.

Summary

Concern for animal welfare requires that we become sensitive to the possibility of animal suffering. This process of sensitisation is not a recent phenomenon and seems in Europe to have its origins in part from scientific understanding of the similarities between animals and humans. There is indeed recent evidence that the belief in animal minds and sentience can positively influence attitudes to animals including fish and invertebrates. Increasingly positive attitudes to animals in Europe have resulted in development of both animal welfare legislation and animal welfare strategies, the latter promoting non-legislative approaches to improving animal welfare. There is also increasing evidence that the concern for animal welfare is becoming a global issue. The World Animal Health Organisation (OiE) has recently responded to the increasing concern for animal welfare by developing an interest in global animal welfare issues including the setting of international standards for trade (OiE 2004). Linked to this socio-political activity has been a realisation that science can provide objective information on animal suffering. From an embryonic state in the 1960s, animal welfare science has grown enormously as a result of increasing research investment from governments and industries affected by animal welfare issues. The central driver behind animal welfare research has been to develop scientific approaches that can be used to ‘represent’ the animal’s perspective. In the past there has been considerable debate over the most appropriate approach for assessing welfare from the animal’s point of view. There are examples where different scientific approaches can be seen to give convergent welfare assessments such as in the case of the parturient sow and farmed mink. However, there are also examples where science has failed to provide a systematically coherent assessment of welfare, which indicates that there is still a need to develop a more robust theoretical basis to animal welfare science.

The FVS meeting provided a number of examples where fish welfare issues are being addressed through the application of approaches previously developed to assess welfare in other species especially in the areas of pain and stress. Given the growing economic importance of fish farming, the increasing public sensitivity to welfare issues in species other than mammals and birds, and the very interesting comparative biology opportunities provided by fish, it seems very likely that research in fish welfare will grow in importance and influence. In the future we can

expect fish welfare research in its turn to provide insights that can be applied more generally to welfare research in other species.

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Chapter 2
Welfare and Fish

Felicity A. Huntingford and Sunil Kadri

Introduction

Fish welfare has become a hot topic. The words crop up in many different contexts: in the primary scientific and veterinary literature, in review articles, in conference programmes, in developing regulations for sustainable aquaculture, in the press and on internet sites. The topic is intellectually challenging for scientists and veterinarians alike, raising as it does complex issues requiring expertise in many different disciplines. It is important in practical terms also, since many human activities potentially affect the welfare of fish and people have strong views on whether and how they should be protected. This chapter covers the questions of what welfare means and whether the differences between fish on the one hand and birds and mammals on the other are sufficient for the concept of welfare to be less applicable to fish. Human activities that potentially compromise the welfare of fish are then discussed, along with whether this matters and how fish welfare can be measured, aspects of which are covered in more detail in several other chapters. The strategies that can be adopted to improve the welfare of fish are also outlined, using aquaculture for illustration, and again touching on topics covered in other chapters, taking into account the implications for welfare of the fact that fish are an extremely diverse group of animals. Finally, the synergistic role of veterinarians and biologists in understanding and protecting fish welfare is considered.

What is welfare?

The concept of welfare is complex and the term difficult to define, because it is used in many different ways by people with very different backgrounds. In addition, scientific uncertainty with regard to whether fish have the capacity for suffering makes welfare a philosophical issue as much as a scientific one (FSBI 2002, Huntingford *et al.* 2006) (see Chapter 1). To summarise and simplify a very extensive literature, most definitions identify an animal as being in a good state of welfare if one of the three following conditions applies:

(1) *The animal can adapt to its environment and is in good health, with all its biological systems working appropriately.*

Some implications and assumptions of such *function-based definitions* are that an animal in poor health (with an impaired immune system, for example) is suffering, by definition, and that an animal that is in good health (growing and/or is breeding well) is experiencing good welfare, again by definition. Problems arise because, while most would agree that good health and proper functioning of bodily systems is necessary for welfare, many would feel that it is not sufficient and that a perfectly healthy animal could still experience poor welfare, for example if a social animal is denied companionship.

(2) *The animal is able to lead a natural life, expressing the same kinds of behaviour as it would in the wild, and is able to meet what are often called its 'behavioural needs'.*

The implications and assumptions of such *nature-based definitions* include the supposition that what is natural is good, by definition, and that animals suffer if they cannot show all their natural behaviour; hens may well need to build nests (Hughes *et al*. 1989) and perhaps sheep need to be frightened from time to time. The concept of behavioural need is complex and much must be known about how a given behaviour is controlled in a given species before it can be concluded that the animals concerned are motivated by the *performance* of that behaviour, rather than by its *consequences*. For example, it may be that salmon swim long distances through the sea because they are tracking food and stop swimming when they find a place to feed. If so, there is no reason to suppose that their welfare is compromised if they cannot migrate, provided they have plenty of food. On the other hand, they may be motivated to swim to new areas regardless of food supply, in which case confinement might well cause suffering, even though fish in cages are able to swim continuously. At present it is simply not known which, if either, is the case.

(3) *The animal is free of negative experiences such as pain, fear and hunger and has access to positive experiences, such as social companionship.*

The implications and assumptions of such *feelings-based definitions* are that a healthy, growing and reproducing animal may not necessarily experience good welfare, that it does not matter if an animal is injured provided that this does not generate negative feelings (on this view fin damage and hooking fish during angling may not necessarily cause suffering), that animals have sufficiently complex mental processes to have positive or negative mental experiences and that these can be identified.

Each of these definitions captures a different, important aspect of animal welfare and, arguably, neither captures everything. For this reason, a different approach to the question of distinguishing between poor and good welfare has been developed and is encapsulated in the famous five freedoms (Mellor & Stafford 2001). This approach recognises five areas, or domains, within which welfare might be

compromised (FAWC 2005). Thus, to guarantee welfare, animals should be free from: (1) hunger and thirst; (2) undue environmental challenge; (3) disease and injury; (4) behavioural restriction (including lack of space); and (5) mental suffering. This approach has been highly influential, forming the broad theoretical framework that underpins much legislation and regulation about the welfare of farmed birds and mammals and, increasingly, of fish. So how different are fish from mammals and birds when it comes to questions of welfare?

Evolution and fish welfare

The physiological and behavioural mechanisms that result in pain and stress are evolved adaptations that enable animals to cope with natural challenges, such as the need to avoid unfavourable habitats, to find food and to avoid being killed by predators. Pain (or nociception as it is more properly called, see Chapter 4) allows animals to detect and avoid localised harmful stimuli. The stress response (see Chapter 3) can be considered to be part of an adaptive strategy for coping with a perceived threat to homeostasis. Concern for the welfare of the animals that humans keep in constrained conditions (for example, on farms or as pets) arises from the fact that these adaptive systems may be activated in contexts where they cannot bring about their natural outcome of either removing the stressor or removing the animal from the source of pain or stress. Much welfare research involves identifying signs that this is the case and finding ways of ameliorating the conditions responsible.

Fish are vertebrates and to a large extent the physiological and behavioural systems that mediate pain and stress are comparable to those of birds and mammals (e.g. review by Wendelaar Bonga 1997). However, fish have had an independent evolutionary history from birds and mammals for more than 350 million years and so are different in ways that are very important for understanding fish welfare. One obvious difference is the fact that the fish live in water and are fully adapted to an aquatic lifestyle. An example of how this relates to welfare is the fact that fish are in intimate contact with their environment through the huge surface of their gills, so water quality is the most critical aspect of their environment (so arguably Domain 2 of the five freedoms is particularly important for fish). Another difference is that fish are ectothermic, so do not need to maintain a constant body temperature. This means that periods of food deprivation that would be lethal to birds and mammals occur naturally and do not necessarily compromise welfare, especially at low temperatures (though they may do so). Arguably therefore, Domain 1 is less important in this group of animals. Finally, systems relating to health and welfare, such as stress physiology and immunology, have been much less intensively studied than have those of mammals. This is because much physiological research is ultimately aimed at understanding human health and function. For example, diseases of fish are poorly understood and most of the cues that are used to identify fear and distress in other vertebrates (e.g. facial and vocal signalling) are simply not available for fish.

With so little known about fish welfare, one might argue that all species are new species.

Our relative lack of information on how fish work is particularly important when it comes to the difficult problem of understanding their cognitive processes and the link between physical welfare and mental suffering in this group (see Chapters 4 and 5). The brain of fishes is relatively small and simple compared to that of birds and mammals and is also organised in a different way. In particular, it does not have the neocortex that in humans is important in mediating the emotional state of suffering. Rose (2002) argues that fish do not have the cognitive ability or brain power to experience suffering, as opposed to simply showing reflex-like responses to adverse conditions. If this is the case, then the concept of fish welfare is meaningless, so it is necessary to ask whether there is an evolutionary cut off for suffering and, if so, whether it falls 'above' fish?

This remains a controversial topic and is discussed fully in Chapters 4 and 5. In brief, it is known that fish are capable of complex behaviour. For example, some form mental maps of their environment and use these for navigation (Reese 1989, Rodriguez *et al*. 1994), while others are able to recognise specific individual companions (e.g. Swaney *et al*. 2001). Also, even though the brain of fish is smaller and organised differently from that of birds and mammals, more and more homologies are being found and anyway, the same function could be performed by different parts of the brain. In addition, recent studies (reviewed in Chapters 4 and 5) suggest that fish do indeed have the capacity to perceive painful stimuli and that these are, at least, strongly aversive. Finally, some species of fish can remember an adverse experience, such as an attack by a predator, for very long periods (Beukema 1970, Czanyi & Doka 1993). At the very least it is clear that experiences such as injury or exposure to predators can be strongly aversive for a fish.

Human activities that potentially compromise fish welfare and why this matters

On the basis of such evidence, much current opinion suggests that fish can probably suffer, even though this is poorly understood and controversial and the experience may well be different from that of birds and mammals. Therefore, fish welfare does mean something and so it is necessary to consider the kinds of impact that human activity may have on the welfare of the fish with which they interact and whether these matter (Huntingford *et al*. 2006). Human impacts range from the very general and 'unintentional' (for example, widespread anthropogenic environmental degradation seriously compromises the welfare of many fish, usually dealt with as a by-product of environmental regulation) to the very specific and 'intentional' (for example, scientific research on fish may impose conditions that compromise welfare in all three senses, dealt with by various national and international legislation and codes of good practice). In between and in some senses more problematic, are

activities such as angling, keeping ornamental fish in domestic or public aquaria (see Chapter 16) (where fish welfare is compromised in the interests of human sport and enjoyment) and activities such as commercial fisheries and aquaculture (where fish welfare is compromised mostly in the interests of providing high quality food for humans).

The question of why it matters that human activities may compromise fish welfare is complex, and far beyond the scope of this book (and of the authors of this chapter), but some relevant issues can be demonstrated by considering the aquaculture industry, where much public attention has been directed. In this context, there are both practical and ethical reasons for taking fish welfare seriously. On the practical front, the aquaculture industry should be (and is) engaging with the welfare of farmed fish because, as good farmers know well, good production and good flesh quality often follow good welfare. Additionally, the public are increasingly and legitimately concerned about the welfare of farmed fish. One consequence is that regulators are responding to this concern by developing regulations and codes of practice with which farmers will have to conform. Another consequence is that fish welfare has become a marketing issue, and there is a price premium for producing fish in a way that maximises welfare.

On the ethical front, the industry should be (and is) engaging with the welfare of farmed fish because there are moral obligations to the animals which are exploited. Welfare biologists and ethicists share the goal of elucidating the relationship between humans and other animals (Fraser 1999). Biologists and veterinarians may be able to identify and possibly measure well-being and suffering. However, it is scholars with a background in philosophy who have the expertise needed to develop frameworks to guide clear thinking on the complex moral issues involved. These include whether and when humans have the right to make use of animals, how to balance the welfare of animals against the needs and wishes of humans, and how to protect the welfare of individuals in conditions where most animals experience good welfare but a few do very badly (e.g. Rollin 1993, Sandøe *et al.* 1997, Heeger & Brom 2001, sections in Huntingford *et al.* 2006). Ultimately, decisions about what is and what is not appropriate lie with legislators and the general public whom they represent.

How to measure fish welfare

In the previous sections, it has been argued that fish welfare is a real concept, that things which humans do, including farming fish, potentially compromise fish welfare and that this matters for both ethical and practical reasons. If these points are correct, there is an onus on all humans to identify when this is the case, and to take appropriate action. This in turn requires that welfare can be measured, since only then can it be known objectively, rather than by guess or intuition, when and how much welfare is compromised.

Table 2.1 Summary of some commonly used measures of welfare and how they relate to different definitions of welfare.

Welfare measure	Definition: Function-based	Definition: Nature-based	Definition: Feelings-based
Physical condition			
• Injury and disease	√		
• Immune system functioning	√		
• Nutritional condition	√		
• Growth	√	√	
• Reproduction	√	√	
Physiological status			
• Metabolic state	√	√	
• Hormones	√	√	
• Brain biochemistry	√	√	√
• Genes on/off	√	√	
Behavioural status			
• Behavioural signs of stress/fear	√	√	√
• Stereotypies		√	√
• Natural repertoire		√	√

As discussed above, welfare is a complex concept, defined in different ways and therefore measured in different ways, depending on the definition one favours (Table 2.1). Most measures used by scientists and other people with an interest in fish welfare (many exemplified by work described in other chapters of this book) concern welfare as defined by function. In this context, physical condition is an important indicator of welfare. This includes the presence or absence of injuries or disease, nutritional status, metabolic status, hormonal status, brain biochemistry, growth, normally-working immune and reproductive systems and the up-regulation of particular genes (Ribas *et al*. 2004). Although there is no simple relationship between stress and welfare, stress responses give us an important part of the picture, especially where several components of the stress response are all influenced in a similar way by the same condition.

Behavioural studies have an important role in welfare research (Mench & Mason 1997), partly in the context of understanding behavioural needs and partly because altered behaviour is an early and easily observed response to adverse conditions. Additionally, choice tests that allow animals to express their natural preferences can help to identify circumstances that may promote or impair their welfare (Dawkins 1998). For example, juvenile damselfish will learn to swim through a simple maze for the opportunity to display aggressively to territorial neighbour damselfish (e.g. Rasa 1971). Though this study was not carried out with fish welfare in mind, the result suggests that the opportunity to exchange aggressive displays may be rewarding and (providing real fights and injury do not occur) promote welfare.

Clearly, there are many possible ways of measuring welfare, all capturing some important aspects of this complex concept, but none giving the whole picture.

Many researchers therefore use more than one measure (perhaps growth and body condition as well as plasma cortisol levels and immunological status as well as food intake), which raises the question of how to combine all these measures to give an overall impression of welfare. There are a number of ways of doing this, including informed common sense, but multivariate statistical tools have been used to produce an integrated measure of welfare, that is consistent with evaluations of welfare by experienced fish farmers (Turnbull *et al*. 2005 and see Chapter 8).

Strategies for improving the welfare of farmed fish

Scientific research into fish welfare has helped to identify practices that do indeed compromise welfare. For example, in the context of aquaculture, welfare research has identified adverse effects of various aspects of husbandry practice, including confinement, inappropriate densities (see Chapter 8), restricted feeding (see Chapter 14), handling (see Chapters 11 and 14), transportation (see Chapter 11) and slaughter (see Chapter 14). What can be done to remove or minimise such effects? Again, using aquaculture as an example, one possible course of action is to ban fish farming altogether. This strategy is favoured by some commentators, but fails to allow for the importance of aquaculture as a source of fish for food when wild stocks are failing and as a source of employment in areas where other employment opportunities are few. Other possible approaches include selecting for farming only those categories of fish that do well in production systems, defining conditions that guarantee welfare and regulating accordingly, developing husbandry systems that maximise welfare, and developing 'litmus tests' for welfare that can be readily applied on farms and regulating accordingly.

Selecting fish that do well in production systems

The long evolutionary history of fish means not just that they are different from birds and mammals, but also that they belong to a very diverse group, so species differ in ways that also have implications for welfare. In considering the needs of existing farmed species or developing new species for aquaculture, species differences have to be taken into account in order to provide favourable welfare conditions. For example, many species of fish form dense schools in the wild, often as a protection against predation, and this is important when assessing the welfare of such species when held at high density in captivity. While in some cases high stocking densities do impair welfare (see Chapter 8), in other cases this is not so and fish appear to do well at extremely high densities, providing water quality is maintained (see Chapter 10). For example, Arctic charr (*Salvelinus alpinus*) are known to display less aggression and to grow faster as stocking density increases (Joergensen *et al.* 1993). In experimental studies, this trend continued beyond the point where the biomass of fish exceeded the water in the tanks, i.e. >500 kg/m^3

(Siikavuopio & Jobling 1995). This example also illustrates the potential for lower stocking densities to impair welfare, and this is not unique to Arctic charr. Atlantic salmon (*Salmo salar*) in tanks show best welfare at intermediate stocking densities (25 kg/m^3) as opposed to lower (15 kg/m^3) or higher (35 kg/m^3) levels (Adams *et al*. unpublished data, and see Chapter 8).

While differences between species are marked, necessitating the customisation of systems and practices to ensure good welfare, differences among fish of the same species can also be very pronounced. Many fish undergo major changes in morphology, behaviour and environmental requirements through their life history. These include many species of cultured fish. For example, Atlantic salmon undergo transformation from freshwater parr (territorial, aggressive fish inhabiting streams and rivers and feeding more or less continuously during the day) to smolts (non-aggressive fish that are particularly sensitive to disturbance while undergoing the process known as smoltification) and then to ocean-going adult salmon (shoaling fish that do not maintain territories and feed in meals). Young cod (*Gadus morhua*) show high levels of cannibalism soon after hatching, but this behaviour is rare in older fish. Husbandry systems and practices need to take such differences into account in order that high welfare standards are maintained throughout the life cycle of the fish being cultured.

In addition to such differences between species and life history stages, fish of the same species and age are not interchangeable, but often show inherited physiological and behavioural variation that adds another dimension of complexity to their welfare needs. In species as diverse as monkeys and sticklebacks, individuals differ strikingly and consistently in their behaviour, and in particular in their readiness to take risks in various contexts, including during fights with conspecifics (e.g. Sih *et al*. 2004). Behavioural differences are often accompanied by differences in physiological response to stress, including lower cortisol responsiveness and higher metabolic rates in aggressive, risk-taking individuals (e.g. Koolhaas *et al*. 1999, Korte *et al*. 2005). Such behavioural and physiological variation (often described as different 'coping strategies') may be inherited and, in evolutionary terms, may be maintained by that spatial or temporal variation in selection regimes, the different behavioural phenotypes performing best in different conditions (e.g. Dingemanse *et al*. 2004).

The coexistence within the same population of individuals with different behavioural profiles has been demonstrated for several species of salmonids, the most intensively farmed fish group (Adams *et al*. 1998, McLaughlin *et al*. 1999, Huntingford & Adams 2005). In a few cases, the physiological processes that underlie differences in risk taking have been identified, strains of rainbow trout (*Oncorhynchus mykiss*) selected for high and low cortisol response to confinement (Pottinger & Carrick 1999) differ in various behaviour (the low responsive strain being more aggressive during staged fights, Pottinger & Carrick 2001) and cognitive function (Moreira *et al*. 2004). Such behavioural and physiological variation potentially has implications for the welfare of farmed fish (staying with aquaculture as an

example), since individuals with different degrees of responsiveness to risk (behavioural and physiological) are likely to be differentially affected by the stresses encountered during intensive production, whether these involve disturbance during routine husbandry or aggressive interactions with conspecifics.

It is certainly the case among salmonids that even a few generations of domestication is sufficient to produce inherited changes in the behavioural profile of farmed stock. Farmed fish show a greater tendency to take risks than their wild counterparts, even when reared in identical conditions (see review by Huntingford 2004), suggesting that production systems do indeed favour bolder, risk-taking fish. The implications for welfare are complex and hard to predict. While domesticated strains may be less responsive to stress generally, if domesticated or low responsive fish are more aggressive (as is sometimes the case), using such strains may create particularly aggressive environments.

Defining conditions that guarantee welfare, and regulate accordingly

Given such uncertainty, the strategy of selecting for intensive aquaculture those species and strains that do well in production systems can only be a partial solution to the problem of promoting the welfare of farmed fish. An alternative approach is to carry out sufficient research to define (for each species and life-history stage) the precise physical and social conditions required for good welfare and to require farmers to keep their fish in these conditions. This is the approach adopted by the UK Farm Animal Welfare Council (FAWC 2005) when 15 kg/m^3 was set as the maximum stocking density for Atlantic salmon, (recognising that more research was needed to define the precise optimum point), and in various codes of good practice for aquaculture. It is attractive, in that the rules would seem to be clear and in principle, easy to apply and monitor. However, because welfare is complex and subject to many different, simultaneously active factors, in reality it is often hard to define conditions in which welfare can be guaranteed (see Chapter 15), even when species and life-history stages are taken into account. For example, welfare in farmed adult salmon is influenced by water quality, disturbance levels and by common husbandry practices, as well as by stocking density (see Chapters 8 and 10). As a result, one can find plenty of fish with good welfare at high densities and plenty of fish with poor welfare at intermediate and low densities (Turnbull *et al*. 2005), so while it might well be appropriate to set limits to stocking density and other aspects of husbandry practice, this alone will not guarantee the welfare of the fish concerned.

Developing husbandry systems that maximise welfare

A different approach is to develop husbandry systems that maximise welfare. These clearly need to be practical and economically viable and a good example of what can be achieved is illustrated in this book in the context of slaughter methods (see

Chapter 14). To give two other examples, adult Atlantic salmon and sea bream (*Sparus aurata*) fed to demand using a feedback control system show less scramble competition and fighting than do those fed the same amount of food in meals (Andrew *et al*. 2002), and salmon parr housed with larger conspecifics fight less and grow faster (Adams *et al*. 2000). So quite simple and relatively cheap changes to husbandry practice could potentially make significant improvements to the welfare of farmed fish.

Develop indices for on-farm assessment of welfare

Each of the strategies outlined above can go some way towards protecting the welfare of farmed fish, but each has its problems. There is a consensus among people from various backgrounds (farmers, veterinarians and welfare scientists) that the best way to ensure good welfare is to formalise what good farmers already do, namely to monitor many aspects of their stock (how they are swimming, what their fins look like, how well they feed and how fast they grow) and build up a picture of their overall welfare. In other words, it is necessary to develop species and stage-specific 'litmus tests' for welfare (validated against established welfare criteria) that can be easily used on farms and to regulate accordingly. Possible indices include body condition, fin condition, colouration, swimming, behaviour during meals, food intake and growth rates. These are discussed in detail in Chapter 15.

The role of veterinarians and biologists in studying and protecting fish welfare

The meeting on which this book was based involved people from a wide variety of backgrounds, all with a strong interest in fish welfare, but with very different perspectives on the topic. It therefore brought together ideas and information from divergent fields including ethics, law, behavioural biology, neurophysiology and neuropharmacology, endocrinology, immunology, aquaculture research, water chemistry and veterinary medicine. This is inevitable, because animal welfare is complex and to identify, measure, understand and protect it requires expertise from all these fields. There is a tendency for researchers to regard their speciality as being of particular value (otherwise why carry on doing it?), but in the context of welfare all these different approaches are necessary. On the one hand, one might argue that fish health is indeed the key to fish welfare, on the grounds that not only is poor health a welfare issue in its own right, but in a sense it is the integrated consequence of many other aspects of welfare (nutrition, stress etc.) and so embraces them all. On the other hand, although good health is necessary for good welfare, it is not sufficient for good welfare in fish or any other animal group.

However, this presents an unnecessarily polarised view, because dialogue between veterinarians and biologists about animal welfare is essential. At the very

least, biologists can provide other indices of welfare that can be used as early warning signs for the onset of poor health. Additionally, biological research is generating better understanding of the factors that compromise fish health, facilitating the development of good preventative veterinary medicine for fish farms. Finally, it will take the combined efforts of veterinarians and biologists to integrate function-based and feelings-based definitions of welfare. Not enough is known about the link between objectively measured physical signs of good or poor health in fish and their subjective feelings. The fish need to 'tell us' about this relationship and biologists and veterinarians are needed to find ways of allowing them to do so.

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Chapter 3

The Stress Response in Fish – Mechanisms, Effects and Measurement

Thomas G. Pottinger

Introduction

The issue of stress is central to most discussions on the welfare of domesticated or intensively farmed animals, including fish (Conte 2004). Stress in this context is perceived as an undesirable consequence of an unsatisfactory regime. For example, is the environment, both physical and social, within which the animal is held inherently stressful? Does the husbandry regime or other procedures to which the animal is subjected elicit a state of stress? Because of the widely held view that there is an inverse association between stress and well-being, the detection of stress has been employed as a tool in assessing the welfare of animals. However, what must not be overlooked is that the stress response is a normal and frequently utilised element of an animal's adaptive repertoire – although activation of the stress response signals that the animal is responding to a challenge, detection of a stress response cannot be considered to be an unambiguous marker of an actual or potential decline in well-being. The context, severity, and duration of the challenge, and of the resultant response, must all be taken into account when assessing whether an adverse outcome is likely for the individual.

The appropriate use and interpretation of physiological markers of welfare status is particularly important when considering fish, which for a variety of reasons offer fewer 'conventional' signals of welfare status. The intention of this chapter is to provide a brief overview of the stress response in fish, its functional role and mechanistic basis, how the response can be measured, and whether detection of a stress response provides information relevant to the assessment of welfare.

What is the stress response?

The adaptive significance of the stress response

The most widely accepted definition of the nature and purpose of the stress response in animals and humans is that which characterises the response as a suite of neuroendocrine events that is activated by a perceived threat and whose purpose is to protect or re-establish homeostasis (Johnson *et al.* 1992). This definition highlights the essential fact that the stress response is a valuable component of the individual's suite of defences. It is not an inherently adverse reaction. Harmful or maladaptive outcomes associated with stress arise because of a mismatch between context and response – for example, the response did not evolve to cope with all the challenges that may be presented by an intensive rearing environment. Furthermore, it is becoming clear that for fish, as is being shown for mammals and birds, focusing on the neuroendocrine response to stress in isolation is a simplification of the complexities of the adaptive processes evoked by exposure to a stressor. Within a population there exists a range of individual 'coping' strategies that encompass variation in the neuroendocrine, physiological and behavioural responses to challenge. These can collectively be considered under the term allostasis – the active maintenance of stability through change (Korte *et al.* 2005, Wingfield 2005). For a consideration of the role of stress in fish welfare, a more simplistic framework remains appropriate.

Terminology

A first hurdle that must be dealt with when discussing the subject of stress is the variation in the terminology employed to describe and discuss stress. For the purposes of this chapter the following terms will be used: a *stressor* is the destabilising stimulus to which the animal responds with a *stress response*, resulting in a state of *stress*.

The stress response in fish

The study of stress in fish has a relatively short history compared to stress research in domestic and laboratory animals, and in a clinical context (see Greenberg *et al.* 2002, for a brief historical overview) but can still be traced back to the late 1960s and early 1970s. It was at this time that fish endocrinologists first adopted techniques that permitted the measurement of steroid hormones at the very low concentrations present in blood (see Donaldson 1981 and references therein). There is now a sophisticated, though incomplete, appreciation of the complex nature of the response in fish and the manner in which it influences, and is itself influenced by, a range of factors, including the immune and reproductive systems, and behavioural and cognitive processes (Bonga 1997, Barton 2002, Øverli *et al.* 2005).

The primary neuroendocrine response to a stressor

The role of the stress response is to enhance the individual's chances of survival when confronted with an immediate threat, real or perceived. The functional importance of the role played by the stress response is evident in the fact that the mechanism has been conserved throughout the vertebrate phylogeny with very little modification. The response is initiated within the limbic system of the brain (Herman *et al.* 2005) although precisely how a potentially damaging or threatening stimulus is assessed and categorised has yet to be defined. Mechanistically, the stress response has two major neuroendocrine components. The first is a rapid activation of the sympathetic nervous system whose endpoint is the release of catecholamines into the blood from the chromaffin tissue (the teleostean homologue of the adrenal medulla: Reid *et al.* 1998, Gallo & Civinini 2003). This is coupled with a slower endocrine cascade that encompasses three tiers of organisation: the hypothalamus, the pituitary and the interrenal tissue (the homologous tissue to the adrenal cortex: Gallo & Civinini 2003). This arm of the response is often referred to as the hypothalamus–pituitary–interrenal (HPI) axis, activation of which culminates in the release of (in teleost fish) the steroid hormone cortisol into the bloodstream (Barton 2002). The elevated levels of catecholamines and cortisol in the blood represent the primary response to a stressor, and these immediately initiate alterations in a variety of tissues that enhance the chances of the animal surviving, overcoming, or avoiding whatever stimulus triggered the response.

The secondary stress response

The core of this phase of the response is a suite of cardio-respiratory changes that in fish include increases in respiratory capacity, heart rate and stroke volume, and blood flow to the gills, accompanied by mobilisation of carbohydrate and lipid reserves (see Pickering & Pottinger 1995). Many of these alterations are attributed to the primary adrenergic response although the precise role of the catecholamines remains to some extent under discussion (Fabbri *et al.* 1998, Perry and Bernier 1999). The role of cortisol in the short-term response to a stressor is less easily defined. While cortisol has some influence, primarily catabolic, on metabolic processes (Andersen *et al.* 1991) and may act in concert with catecholamines (Perry & Reid 1993) its most notable effects tend to be perceived as negative. In particular, the role of elevated cortisol levels in stress-induced immunosuppression is well described (Weyts *et al.* 1999). Although it would appear counter-intuitive that key defence systems should be impaired at a critical time, short-term stress-induced immunosuppression has been rationalised as a necessary 'containment' of processes that would otherwise lead to autoimmune reactions (Nesse & Young 2000). Similarly, the broadly suppressive effects of cortisol on reproductive processes (Schreck *et al.* 2001) and growth (McCormick *et al.* 1998) can be considered to be a diversion of resources away from non-essential activities, until survival of the animal is ensured.

Accompanying these physiological adjustments are alterations in behaviour – for example, increases in alertness and vigilance, escape or freezing behaviour, and in cognitive processes (Johnson *et al.* 1992). However, the precise nature and role of stress-induced behavioural and cognitive changes in fish compared to terrestrial vertebrates remains relatively unknown. It is, however, becoming clear that fish exhibit linked neuroendocrine and behavioural traits that appear to be analogous to the distinct coping strategies identified in other vertebrates (Koolhaas *et al.* 1999, Øverli *et al.* 2005).

The adverse effects of a prolonged stress response

Overall, the net effect of the primary neuroendocrine response to a stressor is to 'prime' the animal for whatever immediate course of action will best ensure its survival. Why, then, is the activation of what is essentially an adaptive and highly beneficial response given prominence in discussions of animal welfare, and considered to be an adverse and undesirable occurrence? The reasons for this apparent paradox relate to the nature of the stressors encountered by captive or intensively farmed animals. It is assumed that the stress response evolved to deal with short-term challenges that could either be overcome or avoided, or to which the animal succumbed. In contrast, captive or intensively farmed animals may encounter situations or events that they perceive as potentially threatening, which cannot be rapidly resolved, but are not inherently lethal. For fish this might include factors such as poor water quality (Pavlidis *et al.* 2003, Ruane & Komen 2003), physical disturbances such as transport (Iversen *et al.* 1998, 2005, Barton *et al.* 2003), or sub-optimal stocking densities and social environments (e.g. Turnbull *et al.* 1998, Ellis *et al.* 2002, Sangiao-Alvarellos *et al.* 2005). Under such circumstances, the HPI axis may be activated intermittently, or continuously, for prolonged periods of time, resulting in a chronically extended stress response that becomes in itself maladaptive and potentially harmful (Barton 2002). Outcomes associated with exposure to unalleviated stressors, or frequent encounters with acutely stressful events include growth suppression, reproductive dysfunction, and loss of immunocompetence leading to enhanced susceptibility to pathogens and parasites (see Bonga 1997 for references).

Detection and measurement of stress in fish

Advances in endocrinology, biochemistry and molecular biology mean that it is now possible, in principle, to detect and quantify changes in almost every element of the primary stress response in fish. Because of the relevance of stress-related studies to aquaculture (Pickering 1992) and because of the ready availability of rainbow trout (*Oncorhynchus mykiss*) as an experimental animal in both Europe and the United States, this species together with other members of the Salmonidae, has become one of the best documented and most studied, and a continuing platform for cutting

edge science (Thorgaard *et al.* 2002). However, in recent years the range of species studied has expanded significantly and increasingly, data are becoming available for almost all the major taxonomic groups.

Measuring components of the primary stress response

A significant problem that faces researchers seeking to investigate aspects of the stress response in fish is the fact that experimental conditions and procedures are themselves often potentially stressful. Therefore, great care must be taken in designing protocols that avoid inappropriate activation of the systems under study. This problem is exemplified by the difficulties inherent in measuring the pivotal element of the primary neuroendocrine stress response, the rapid elevation of blood catecholamines. The rapidity of the adrenergic response counters attempts to obtain meaningful measurements – any disturbance of the fish associated with sampling tends to activate the response. This is not an issue for most of the components of the HPI axis because there is enough of a lag between activation of the response and actual change in the endpoint being measured. However, alterations in circulating catecholamine concentrations occur extremely rapidly, so information on blood catecholamine dynamics during stress in fish are limited and have been collected using invasive procedures such as indwelling catheters (Gingerich & Drottar 1989). This renders blood catecholamine levels an unsuitable index for routine use in detecting and quantifying the stress response in fish.

The neurotransmitters that influence, and are influenced by, the stress response such as those comprising the serotonergic system, can be measured directly in specific areas of the brain by high performance liquid chromatography (HPLC: Øverli *et al.* 2001) while alterations in the elements of the endocrine HPI cascade can be assessed at the level of gene expression by measuring changes in the amounts of specific mRNA present at the site of production (corticotropin releasing factor (CRF): Doyon *et al.* 2005), or by direct measurement of the peptides themselves in the blood (adrenocorticotropic hormone (ACTH): Sumpter *et al.* 1986) using highly specific radioimmunoassays. The levels of other pituitary hormones whose secretion is altered during stress can also be determined in this way (e.g. prolactin (PRL): Pottinger *et al.* 1992a; somatolactin (SL): Rand-Weaver *et al.* 1993). The most widely used indicator of HPI activation in studies concerning stress in fish is blood levels of the steroid hormone cortisol. Cortisol can be measured using a number of analytical techniques but radioimmunoassay (e.g. Pottinger & Carrick 2000) and ELISA (e.g. Tintos *et al.* 2006) have been the most widely adopted methods. The use of cortisol as an index of stress will be discussed in greater length below.

Measuring components of the secondary stress response

The actions of corticosteroids and catecholamines at their respective target tissues result in a wide range of alterations in physiological status, many of which are

susceptible to direct measurement. Increases in levels of blood-borne metabolites such as glucose and lactate, depletion of reserves such as hepatic glycogen, and alterations in metabolically active enzymes are readily measured by standard colorimetric assays (e.g. Trenzado *et al.* 2003) and blood samples can if necessary be processed and assayed away from the laboratory using portable apparatus with adequate levels of sensitivity (Wells & Pankhurst 1999). Alterations in haematological indices, such as the number of circulating lymphocytes, can be determined by direct inspection of stained blood smears (e.g. Pottinger *et al.* 1994) or by flow cytometry (Morgan *et al.* 1993). Changes that are less accessible to measurement, but nonetheless informative, include alterations in target-tissue corticosteroid receptor abundance (Pottinger 1990) and modifications in gene expression (Nagano *et al.* 2003). Changes at this level of organisation are delayed in relation to the onset of the response and are most likely to be evident during prolonged, or chronic, activation of the HPI axis. Finally, behavioural modifications following exposure to stressors can be quantified in fish (e.g. alterations in predator avoidance behaviour: Olla *et al.* 1992) but such measurements are accompanied by problems of observation and interpretation.

The tertiary stress response

The adverse consequences of exposure to continued unresolved chronic stressors are collectively referred to as the tertiary stage of the stress response (Barton 2002). These effects are likely to be highly visible and easily detected, and clearly represent a situation in which welfare has been compromised. Among the indicators of a tertiary stage stress response are a reduction or cessation of growth, a decline in condition factor, increased incidence of disease (bacterial, fungal, viral, ectoparasites) and deteriorating reproductive status (gamete quality, fecundity etc.). The occurrence of one or more of these conditions signals the need for immediate remedial action.

Cortisol as an index of stress: measurement of hormone concentrations

A number of criteria must be satisfied when selecting a workable and informative measure of stress in fish. The marker must have some diagnostic value, being informative as to the current status of the fish, and should also ideally be a relevant and reliable indicator that is specific to the stress response. The index should preferably be characterised by a stable and unambiguous baseline against which change can be readily detected. It should preferably be accessible via a non-destructive route not requiring the removal of tissue samples. Ideally, it should be measurable without recourse to expensive and complex procedures. The single index that comes closest to satisfying these requirements is the steroid hormone cortisol.

Baseline blood cortisol levels in unstressed fish are normally low relative to the levels achieved during stress, although the absolute concentrations under each condition can vary with species. Elevation of blood cortisol tends to be associated primarily with exposure to stressors although there are some limited exceptions to this (e.g. pre-reproduction in female salmonids: Pickering & Christie 1981). Detection of stress-induced elevation of blood cortisol has significant diagnostic content because of the causal link between cortisol and many of the adverse outcomes of exposure to chronic stressors (Mommsen *et al.* 1999). Cortisol is detectable using very precise and sensitive assay systems (radioimmunoassay (RIA); enzyme-linked immunosorbent assay (ELISA)) and it can be measured in a variety of compartments. Finally, there is a wealth of published data on cortisol in fish (Barton & Iwama 1991).

Cortisol in the blood

From a research perspective, the most meaningful measurement of cortisol levels is that obtained in the blood. This is the compartment that is most immediately reflective of the secretory activity of the interrenal tissue and the concentration that most closely equates to that to which the target tissues are exposed. Blood samples can readily be obtained using a hypodermic needle and syringe (cannulation will not be considered as a viable alternative for routine use because of the complexities of the procedure) from a lightly sedated fish. Samples can be obtained by heart puncture, from the caudal vein or artery, or from the cuvierian ducts (posterior cardinal veins). The constraint on this approach is that it is critical that the time elapsed between initial disturbance of the fish, by for example netting from a holding tank, and removal of the blood sample is less than five minutes. Otherwise, as noted above, the disturbance associated with the act of sampling will initiate a stress response, or possibly augment an existing response (Pickering *et al.* 1982). If sampling disturbance cannot be eliminated, alternative approaches to assessing cortisol levels can be adopted.

Cortisol in the bile

Cortisol, in common with all steroid hormones, is inactivated and cleared from the body via hepatic biotransformation processes. This results in the accumulation of cortisol metabolites and their conjugates within the gall bladder. The levels of these metabolites has been shown to be extremely high in fish exposed to a chronic stressor (Pottinger *et al.* 1992b) but accumulation of cortisol and cortisol metabolites in the bile exhibits a lag time following the onset of changes that are detectable in the blood. In situations where blood samples cannot be obtained rapidly after the onset of the sampling procedure, the measurement of cortisol and its derivatives in the bile is one means by which short-term alterations in HPI activity can be excluded. Although the analytical methods employed to measure cortisol (RIA and

ELISA) are highly specific when used with blood, when used to assay tissues or fluids other than blood they may also detect derivatives of cortisol, particularly those that are present at high concentrations. The precise identity of the cortisol metabolites present, and the extent to which they cross-react with the assay antibody, is usually unknown. Therefore, when using an antibody-based assay system to measure cortisol in compartments other than blood, it is advisable to consider that all immunoreactive cortisol-like steroids are detected.

Cortisol in whole body homogenates

In situations in which the fish is too small to obtain a blood sample, cortisol levels can be determined in whole-body homogenates. For this approach the fish is humanely killed before being homogenised in a suitable vehicle. Immunoreactive cortisol-like steroid levels are then measured in the supernatant of the homogenate. This approach lacks the precision of direct measurement of cortisol in the blood because the result integrates cortisol (and its derivatives) present in all body compartments, including the blood and gall bladder. However, the profile with time of cortisol-like steroids detected in whole body homogenates has been shown to broadly approximate that of cortisol in the blood during exposure of trout to a prolonged stressor (Pottinger & Mosuwe 1994) and the method has proved useful for monitoring the stress response in small fish such as sticklebacks (Pottinger *et al.* 2002) and zebra fish (Pottinger & Calder 1995).

Non-invasive measurement of cortisol – detection in water and faeces

A large proportion of circulating cortisol is actively cleared from the circulation by bioconversion and excretion via the bile. However, some steroid conjugates are also excreted via the urine and a considerable quantity of free steroids are lost passively from the blood during its passage through the gills (Vermeirssen & Scott 1996, Ellis *et al.* 2005). Steroids pass freely through cell membranes and the same structural characteristics that ensure that the gills are efficient at removing oxygen from water also ensures that steroids readily exit the fish down a concentration gradient. This efflux is exacerbated for cortisol because, in contrast to mammals, fish do not appear to possess a blood-borne high-affinity transcortin-like binding protein. Recent studies have shown that meaningful information concerning the endocrine status of a population of fish can be obtained by direct measurement of cortisol in the water, effectively integrating the cortisol status of a large group of fish simultaneously, in a completely non-invasive manner (Ruane & Komen 2003, Ellis *et al.* 2004, Lower *et al.* 2005). The two most obvious drawbacks of this approach are the loss of information concerning individual variation, which in many cases may not actually be necessary, and the requirement for an enclosed volume of water with known inputs and outputs, which may be an issue in attempting to apply

this technique to fish in open cage culture. However, an alternative non-invasive method for the detection of the stress status of fish has been reported recently which to some extent remedies these shortcomings. The measurement of steroid hormones and their conjugates in faecal material has long been employed to provide information on the endocrine status of free-living terrestrial animals that are not accessible for the recovery of blood samples (Millspaugh & Washburn 2004). This methodology has successfully been applied to fish (Karsten & Turner 2003, Turner *et al.* 2003) and offers promise as a means of obtaining more detailed information on corticosteroid activity at an individual level. A further advantage of this method is its applicability to fish outside the confines of a tank – information can be obtained from free-swimming or cage-confined fish. Both these non-invasive approaches to the assessment of endocrine status in fish offer great potential as informative indices of well-being for fish in a range of environments.

Cortisol as an index of stress: modifying factors

Although cortisol concentration, in whatever compartment it is measured, is a reliable indicator of stress status in fish, its use is not without complicating factors. To highlight some issues that must be borne in mind if utilising cortisol in a diagnostic capacity, some of these will be briefly discussed. The stress response, although overall a predictable and reproducible event is subject to modulation by a number of factors, both intrinsic and extrinsic to the fish. Developmental stage is a factor that must be considered. In some species, the juvenile fish is 'stress competent' immediately following hatch (tilapia, *Oreochromis mossambicus*: Pepels & Balm 2004) whereas in other species there may be some delay before the juveniles are capable of responding to disturbance with an elevation of cortisol. This may be as little as a week (chinook salmon, *Oncorhynchus tshawytscha*: Feist & Schreck 2001; yellow perch, *Perca flavescens*: Jentoft *et al.* 2002) or may be longer (brown trout (*Salmo trutta*) and rainbow trout: Pottinger & Mosuwe 1994). As adults, during sexual maturity, the responsiveness of the male to a stressor may be markedly attenuated relative to that of the female (rainbow trout and brown trout: Pottinger *et al.* 1995, Pottinger & Carrick 2000). The magnitude of the response, and its dynamic in both sexes is strongly influenced by water temperature, with a much reduced and slower response in winter months compared to summer months (Sumpter *et al.* 1985). Habituation or acclimation of the stress response may occur when fish are repeatedly exposed to a stressor that is not inherently harmful (Pickering & Pottinger 1985). A further source of variation is introduced by inter-individual differences in responsiveness to stressors. While the magnitude of the cortisol response seems to be a stable individual trait, there is significant inter-individual variation within a population (Pottinger *et al.* 1992c) and between genetically distinct lines (Pottinger & Moran 1993). In addition to this within-species variation in the characteristics of

the cortisol response to stress, there is also between-species variation. For example, although no systematic survey has yet been undertaken, there is a quite profound difference in the magnitude of the cortisol response to similar stressors between salmonid fish and cyprinid fish with the latter exhibiting a more pronounced increase (Pottinger *et al.* 1998, 2000).

Ameliorating stress in farmed fish: selective breeding for reduced stress responsiveness

The most obvious approach to limiting the stress associated with a particular regime is to reduce the frequency, duration and severity of stressors to which fish are exposed. This is most effectively achieved by ensuring that husbandry procedures are as benign and welfare-centric as is possible. However, this may not be an option if changes in management practices to reduce the degree of stress are subject to significant economic and practical constraints. It must also be borne in mind that while most species of economic significance to conventional agriculture are fully domesticated, resulting in animals with a tolerance of environments and stimuli which would be stressful to wild stock, farmed fish, particularly newly exploited species, are not far removed from the wild state and cannot be considered domesticated (Mignon-Grasteau *et al.* 2005). One obvious approach to reducing the stress experienced by fish under intensive rearing conditions is to enhance or accelerate the process of domestication.

Domestication is a consequence of a long-term and continuing selective breeding programme, both deliberate and inadvertent. If an enhanced tolerance of stressful events or conditions is a factor in domestication then the domestication of economically important fish species may be assisted by selectively breeding for reduced responsiveness to stressors (Pottinger & Pickering 1997, Pottinger 2000). The rationale for this approach lies with the assumption that by reducing the magnitude of response to conditions that elicit a stress response, the longer-term cumulative effects of stress on the performance and well-being of the population will be reduced.

The magnitude of the stress-induced elevation of plasma cortisol is a key aspect of responsiveness to stressors that is also accessible to selection. By reducing the amount of cortisol that reaches target tissues during unavoidable episodes of stress, the adverse effects of such experiences should be broadly ameliorated. The magnitude of post-stress plasma cortisol elevation in rainbow trout is a stable trait within individual fish (Pottinger *et al.* 1992c) and both semi-quantitative (Pottinger *et al.* 1994) and quantitative (Fevolden *et al.* 1999, Pottinger & Carrick 1999, 2001a) studies have demonstrated that post-stress plasma cortisol elevation has a moderately high heritability ($h^2 = 0.4$–0.6). These factors have facilitated the production by selective breeding of two lines of trout (high-responding (HR);

low-responding (LR)) that exhibit divergent cortisol responses to a standardised stressor.

Investigation of these lines has shown that physiological and neuroendocrine differences arising from selection for either high or low activity of the HPI axis during stress (Øverli *et al.* 2001, Pottinger & Carrick 2001b, Trenzado *et al.* 2003) are accompanied by divergent behavioural characteristics (Øverli *et al.* 2005) and cognitive attributes (Moreira *et al.* 2004). The possibility that these differences in behaviour are the result of processes not directly arising from selection for stress responsiveness cannot be completely ruled out. However, these studies strongly suggest that the two selected lines correspond to two distinct combinations of physiological and behavioural strategies for dealing with stressful circumstances, analogous to the proactive and reactive coping strategies found in mammals (Koolhaas *et al.* 1999). The HR rainbow trout line exhibits a combination of neuroendocrine and behavioural characteristics that resemble the reactive model, while the LR trout line corresponds more closely to the proactive model (Pottinger & Carrick 2001a, Øverli *et al.* 2002, 2005, Schjolden *et al.* 2005).

Despite extensive characterisation of the behavioural and physiological attributes of the two lines, the functional significance of possessing one or other trait (high- or low-responsiveness to stressors) under conditions that resemble more closely the commercial aquaculture environment has yet to be definitively established. Certainly, in co-culture the LR line outperforms the HR line for reasons that appear to be related to the behavioural characteristics of the lines and it has been suggested that selection on single traits, like stress responsiveness, may result in unpredicted outcomes in aquaculture environment (Pottinger 2006). The value of selective breeding for the enhancement of single traits associated with stress responsiveness may lie in providing a means to identify the genetic basis of the trait for eventual inclusion in multi-trait marker assisted selection schemes, rather than direct exploitation in themselves.

Conclusions

Fish possess a neuroendocrine stress response that is of clear adaptive value in the short-term but when activated for prolonged periods may be harmful, with definite implications for the welfare of the fish. Many intrinsic and extrinsic factors modulate the response and its magnitude varies between individuals and is a heritable trait. There are qualitative and quantitative inter-species differences in the stress response. Measurement of indices of stress, together with other indicators of well-being, can inform assessments of the welfare status of fish. Cortisol is the most informative and accessible marker of stress in fish and it can be measured in a wide variety of contexts. Manipulation of the magnitude of the cortisol response to stress by selective breeding may offer a route by which the deleterious effects of stress in an aquaculture environment can be countered.

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Chapter 4
Pain and Fear in Fish

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Introduction

When considering the welfare of any animal it is important to know how our actions and the procedures that we subject them to affect their well-being. There is increasing public concern over the welfare of farmed animals whereby it is thought that animals used by consumers should not have suffered at any stage of the rearing or harvesting practices. This ideal is also shared by international regulatory bodies such as the Canadian Care Council, US Department of Agriculture and the UK Farm Animal Welfare Council. Therefore, it is vital that we understand whether animals are capable of suffering and also if the procedures we subject them to cause such suffering. There has been significant controversy in the global media about the capacity of fish to perceive pain and consequently suffer. Fish are subject to a number of invasive practices such as hooking in angling, surgery in experimentation, damage during trawling in commercial fisheries and also during intensive fish farming (e.g. handling, size grading, vaccination, disease, slaughter). This chapter will address the capacity for suffering by examining two specific forms of suffering in detail, specifically the capacity to experience pain and fear. Presented is a review of the evidence for the existence of pain and fear in fish and, therefore, the possibility that fish should have similar welfare considerations to those of higher vertebrates.

Concepts of animal welfare have traditionally been applied to species which are considered to have the ability to experience pain, fear and suffering and as such have been associated with species with an apparently higher level of cognition when compared to fish. While scientific debate still continues as to whether fish have the neural capacity for awareness, fear and pain, there can be no doubt that practices in aquaculture, commercial and recreational fishing, and scientific research do provide potentially painful and fearful situations (Conte 2004). It is unlikely that animals with a different brain structure to humans would experience anything like the emotions that humans feel when experiencing pain and fear. However, if an animal experiences suffering or discomfort, the nature of the pain or fear they perceive is no less important.

Nociception is the detection of potentially injurious stimuli and is usually accompanied by an immediate reflex withdrawal response away from the noxious

stimulus. Crucial to survival in all animals, nociception in humans can give rise to pain, which is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (IASP 1979). Although all animal groups are thought to possess nociceptors, much of the research on nociception has focused on understanding human pain. As such there is a wealth of information regarding nociception in mammalian systems (Lynn 1994). More recently non-mammalian vertebrates, amphibian and avian models, have been extensively studied (e.g. birds: Gentle 1992; amphibians: Willenbring & Stevens 1995). In comparison, nociception and pain are relatively under-explored in fish. However, recent studies have addressed these important questions and have not only identified and described the properties of peripheral receptors that preferentially detect noxious stimuli (nociceptors) in fish (Sneddon 2003a) but also scrutinised the behavioural responses to a potentially painful event (Sneddon 2003b, Sneddon *et al.* 2003a, b).

Fear may be defined as a behavioural and physiological response to perceived threat or danger and functions to increase evasion from these threats and ultimately increase survival (Jones 1997, Chandroo *et al.* 2004). The neural system underlying fear in higher vertebrates has been well studied and much of this work relates to the behavioural paradigm termed fear conditioning. Here, rather than describing the mechanisms by which the subjective states of fear arise and are experienced, the fear system has been treated as a set of processing circuits that detect and respond to danger (LeDoux 2000). Consistent behavioural and physiological responses to potentially fearful stimuli, similar to those seen in higher vertebrates, have been described in fish. Also, the neurobehavioural basis for fear conditioning in fish appears to be dependent upon cognitive mechanisms and homologous brain regions similar to those of higher vertebrates (Chandroo *et al.* 2004).

While some reviewers have argued that fish lack essential brain regions or any functional equivalent, making it untenable that they can experience pain and fear (Rose 2002), others suggest that there is anatomical, physiological, and behavioural evidence that make it conceivable that nociception in fish is consciously experienced and that they have some capacity to experience pain and fear (Chandroo *et al.* 2004). Empirical evidence from studies designed to examine pain and fear in fish has also produced significant evidence for the ability of fish to experience these two forms of suffering.

The question of sentience and consciousness

Satisfactory definitions of sentience and consciousness are lacking due to the general disagreement on the meaning of these terms between scientists. Here, we define sentience as the ability to detect and respond to external stimuli. Consciousness can be defined as a sense of 'I' and how 'I' relate to the world (Beckoff & Sherman 2004). A plethora of studies have demonstrated that fish can detect and respond

to external stimuli (e.g. nociceptors, receptors that can detect noxious stimuli, are present and fish react behaviourally and physiologically to their stimulation), but the important issue is whether the fish are conscious (i.e. do they know that they are in pain?). Do fish have conscious thought whereby they recognise their condition and form thoughts about it in their mind? Fish have previously been denied the welfare considerations given to higher vertebrates on the basis that they do not demonstrate the cognitive characteristics of conscious beings and, therefore, do not consciously experience the motivational affective states of pain and fear. It is impossible to measure emotion directly in an animal, therefore the indirect evidence used to establish such conscious motivational affective states must come from neuroanatomy, neurophysiology, and particularly behaviour (Duncan 2002). Specific evidence for the experience of both pain and fear will be discussed below. The issue of consciousness or sentience has relevance to both states and so will be discussed in general terms here. Particular types of behaviour are thought to be indicative of an animal's ability to form and act upon structured, internal neural representations of its internal and external environment. This state of 'primary consciousness' is only thought to have been achieved in species that have nervous systems that have attained a sufficient level of complexity during evolution (Arhem & Liljenstrom 1997, Shettleworth 2001). The cognitive abilities and neuroanatomical features of an animal may, therefore, be used to assess consciousness and sentience.

Behaviour

Chandroo *et al*. (2004) reviews a number of studies on different fish species that provide evidence to suggest that fish possess the cognitive abilities that indicate that conscious cognition has evolved in fish (see Chapter 5). Studies on learning in a number of different species seem to indicate that teleost fish are capable of mental construction that can direct behaviour in flexible and adaptive ways. Species including paradise fish, *Macropodus opercularis* (Topál & Csányi 1999), Siamese fighting fish, *Betta splendens* (McGregor *et al.* 2001), and rainbow trout, *Oncorhynchus mykiss* (Johnsson & Akerman 1998) seem to be able to extract information from external stimuli (e.g. through observational learning, see Brown & Laland 2003) and in combination with memories of former experiences, subsequently use that information to direct their future behaviour. This ability seems particularly evident in cases where fish appear to modify their aggressive behaviour during conspecific agonistic encounters according to both observations of previous encounters between two other fish and memories of previous first hand competitive experiences (Johnsson & Akerman 1998, Oliviera *et al*. 1998, McGregor *et al*. 2001, Chandroo *et al*. 2004). We do not argue that fish have an identical emotional experience to humans but we do suggest that perhaps consciousness is on a phylogenetically sliding scale in vertebrates and that fish may experience a more primitive form of pain and fear (Beckoff & Sherman 2004). However, it is impossible to know exactly what

a fish experiences unless one has actually been a fish and, therefore, it is best to give fish the benefit of the doubt if they possess the neural apparatus to detect and react to pain and fear and show similar behavioural responses to higher vertebrates, including humans.

Neuroanatomy and neurophysiology

All vertebrates develop a forebrain and midbrain (i.e. telencephalon and diencephalon regions). Neural pathways that connect to various forebrain structures are of fundamental importance to consciousness and the perception of pain and fear (Willis & Westlund 1997). Over the course of evolution, the pallium (the grey matter that covers the telencephalon) has thickened to various extents in different classes of vertebrates, and in mammals it consists of a laminated structure, the cerebral cortex (Striedter 1997). The most highly evolved vertebrates, humans and primates, have the most developed cortex with the evolution of the neocortex. Unlike mammals, the pallium of the majority of modern fish species is unlaminated, however there is evidence to suggest it has developed into a highly differentiated structure with respect to the processing of sensory information (Bradford 1995, Butler 2000). The telencephalon in fish is thought to be the location of several key brain structures that are functionally homologous to those associated with pain and fear in higher vertebrates (Bradford 1995, Chandroo *et al* 2004, Portavella *et al*. 2004). Deficits caused by ablation or lesion of structures in the limbic system of mammals are similar to those seen in fish following similar procedures on the telencephalon (Portavella *et al.* 2002). In higher vertebrates, the hippocampus is involved in memory and learning of spatial relationships. Lesions to this area produce deficits in spatial and associative learning, and ablation of the telencephalon of fish produces similar deficits (Salas *et al*. 1996a, b, Lopez *et al*. 2000a, b). In mammals, the amygdala has long been known to be important in arousal and emotions, particularly the fear response (Carter 1996, Maren 2001). The amygdaloid complex in fish is located in the telencephalon (Butler 2000) and electrical stimulation of this region produces changes in aggression similar to those observed following amygdalar lesions or stimulation in higher vertebrates (Bradford 1995, Portavella *et al.* 2002). The hypothalamus in fish is thought to perform a similar function to that in other vertebrates, amongst other things being responsible for the integration of both internal and external signals including those originating from those telencephalic areas that have been implicated in emotional learning (Fox *et al*. 1997, Portavella *et al*. 2002, Chandroo *et al*. 2004).

Telencephalic dopaminergic neurons are thought to be particularly important with regard to reward behaviour and learning in both fish and higher vertebrates (Chandroo *et al.* 2004). Fish also appear to possess similar dopaminergic systems to those implicated in the control and expression of behaviour that results from emotion in higher vertebrates, and these systems are thought to mediate similar reward mechanisms including fear and aggression (Chandroo *et al*. 2004).

In summary, behavioural, neuroanatomical and neurophysiological studies would appear to suggest that at some level, states such as fear and pain might be consciously experienced by fish.

Pain

As animals are unable to express pain directly, a number of criteria have been defined to provide a guide as to whether an animal might be capable of experiencing pain (Bateson 1991). The inability to communicate verbally does not negate the possibility that an individual is experiencing pain (IASP 1979). The first requirements to determine whether nociception can occur are the possession of nociceptors along with the presence of endogenous opioids, opioid receptors and enkephalins. Further criteria for the evidence of pain stipulate that specific brain structures involved in pain processing and the pathways leading to higher brain structures must also be present. The ability of analgesics to reduce nociceptive responses, and whole animal responses such as avoidance learning are also crucial. Finally, responses to a noxious event must be more than a simple reflex and must be indicative of suffering with a suspension of normal behaviour. Each of these areas shall be taken in turn to assess how well fish fulfil these criteria and how they compare to the nociception and pain systems of higher vertebrates.

Nociceptor anatomy

Generally, nociceptors are free nerve endings in the periphery, internal organs and viscera and are usually of two fibre or nerve types: small myelinated A-delta fibres with high conduction velocities and smaller unmyelinated C fibres with slower conduction velocities (Lynn 1994). A recent study on the rainbow trout has shown that both fibre types are present in the trigeminal nerve (Sneddon 2002). The size range of both the C fibres and A-delta fibres found in rainbow trout matched those found in higher vertebrates. However, in the fish trigeminal – a nerve that conveys nociceptive information from oral and facial areas in higher vertebrates – A-delta fibres comprised 33% of total fibre type and C fibres comprised 4% of total fibre type. This is in contrast to higher vertebrates such as amphibians, birds and mammals where C fibres comprise 50–65% of total fibre type, outnumbering A-delta fibres 4 to 1 (Lynn 1994).

The greater risk of injury in the terrestrial environment when compared to the aquatic environment may provide an explanation for this reduced number of C fibres in fish relative to the high number found in mammals. While animals that have evolved to live on land must contend with gravitational forces, noxious gases and extremes of temperature, aquatic animals are subjected to smaller fluctuations in temperature, gravity, and chemicals through buoyancy, dilution, and the general thermal stability provided by the underwater environment. Therefore, teleost fish

may not have devoted as much neural wiring to a nociceptive system as the terrestrial vertebrates, who have a much more comprehensive system to deal with the increased risk of damage. This hypothesis remains to be tested (Sneddon 2004).

Elasmobranch fish, the sharks, skates and rays, have been shown to possess A-delta fibres (e.g. stingray, *Dasyatis sabina*; spotted eagle ray, *Aetobatus narinari*; cownose ray, *Rhinoptera bonasus*; longtailed ray, *Himantura* sp.; Coggeshall *et al.* 1978, Leonard 1985, Snow *et al.* 1996), however, they show a lack of C fibres. Unmyelinated fibres that could be C fibres were found in the shovelnose ray, *Rhinobatos batillum*, and the black-tip shark, *Carcharhinus melanopterus*, however the researchers suggest that myelination was incomplete in the small specimens used (Snow *et al.* 1996). Leonard (1985) also states that only the sensory nerves that enter the dorsal root ganglion of the spinal cord contain less than 1% unmyelinated fibres but more are found in the cranial nerves; but whether the trigeminal nerve was included in the cranial nerves is not divulged in the paper. The lamprey, *Petromyzon marinus*, the oldest living predecessor of the fishes, has only unmyelinated fibres (Matthews & Wicklegren 1978).

Nociceptor properties

Nociceptors can be classified into various types according to their physiological properties, however, they are generally characterised by their slowly adapting response to mechanical stimulation, their response to noxious heat (>40°C) and in some cases they also respond to noxious chemicals (Lynn 1994). Electrophysiological recordings in the lamprey, *P. marinus*, found slowly adapting receptors on the head that only responded to noxious heat and damaging stimuli and, therefore, may possibly provide nociceptive function (Matthews & Wicklegren 1978). Similar studies have failed to find nociceptors in elasmobranchs (Coggeshall *et al.* 1978, Leonard 1985). Studies on the trigeminal nerve (containing both C fibres and A-delta fibres) in rainbow trout showed the presence of nociceptors on the head that were innervated by this nerve. Five types of receptors were found and of these, 35% were nociceptors and were located on the head, lips and opercular area of the trout (Figure 4.1; Sneddon 2003a). A high proportion of these nociceptors were classed as polymodal as they responded to both noxious heat over 40°C and the application of a noxious chemical, 1% acetic acid.

The majority of the 22 nociceptors found on the rainbow trout head were fast conducting A-delta fibres, with only one being a slower conducting C fibre. Therefore, the majority of polymodal nociceptors were A-delta fibres. However, in higher vertebrate skin, C fibres usually act as polymodal nociceptors (Lang *et al.* 1990). In mammals, polymodal A-delta fibres are usually found in oral mucosa (Toda *et al.* 1997), skeletal muscle (Kumazawa & Mizumura 1977) and visceral organs (Kumazawa & Mizumura 1980, Haupt *et al.* 1983). The polymodal nature of these A-delta nociceptors may be due to the aqueous environment in which they

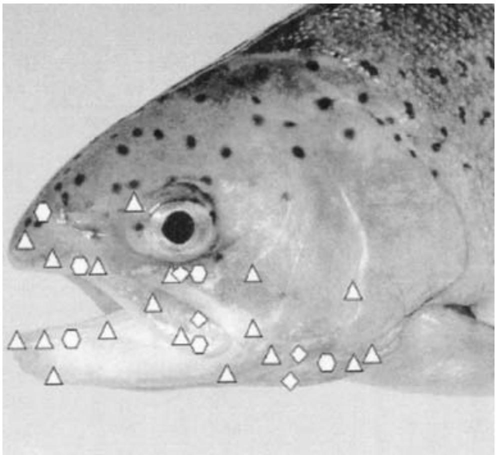

Figure 4.1 Location of the nociceptors and chemical receptors on the head of the trout. △ = polymodal nociceptor, ◇ = mechanothermal nociceptor, ⬡ = mechanochemical receptor (Reprinted from Sneddon *et al*. (2003) *Proceedings of the Royal Society of London B*, **270**, 1115–21, with kind permission from The Royal Society of London.)

function, since they come into contact with a mixture of mechanical, chemical and thermal stimulation. The high levels of the polymodal nociceptors in fish skin may, therefore, also be related to the aquatic environment they inhabit (Sneddon 2003a).

The properties of the nociceptors seen in rainbow trout are similar to those seen in higher vertebrates. For example, the diameter of the receptive fields ranged from 1.6 to 9 mm which is a similar diameter found in birds (Gentle & Tilston 2000) and mammals (Toda *et al.* 1997); the fish nociceptors have large, broad action potentials with slow depolarisation as also seen in mammals (Gallego 1983, López de Armentia *et al.* 2000); and finally the conduction velocity of the fish nociceptors are within the mammalian A-delta and C fibre range (Lynn 1994).

However, while a minimum pressure of 0.6 g is required to stimulate mammalian skin nociceptors (Lynn 1994) some of those found in the rainbow trout head were stimulated by less than 0.1 g (Sneddon 2003a). Thresholds this low are, however, seen in corneal nociceptors of mammals (Belmonte & Gallar 1996). The electrophysiological properties of nociceptors found on the mouse cornea (López de Armentia *et al.* 2000) and those found on the head of the snake (Liang & Terashima 1993) are similar to those found on the trout head (Table 4.1). Characteristics such as rate of firing and action potential properties, although slower are similar if differences in body temperature are accounted for. That teleost fish share common nociceptor electrophysiological properties with both a reptile and a mammal, suggests that these are primitive characteristics that may have evolved in a predecessor of the teleost group. The electrophysiological properties of nociceptors in the agnathans therefore warrants further investigation.

Table 4.1 Electrophysiological properties of A-delta nociceptors in a fish (Sneddon 2003a), a snake (Liang & Terashima 1993) and a mouse (López de Armentia *et al.* 2000). Mean values are shown for conduction velocity, action potential (AP) amplitude and duration, afterhyperpolarisation (AHP) amplitude and the maximum rate of depolarisation (dV/dtmax). Reprinted from López de Armentia *et al*. (2000) *Neuroscience* **101**, 1109–15, with kind permission from Elsevier and from Liang & Terashima (1993) *Journal of Comparative Neurology*, **328**, 88–102, with kind permission from John Wiley & Sons Inc.

	Fish	Snake	Mouse
Conduction velocity (m/s)	0.7–5.5	3.8	0.7–5.7
AP amplitude (mV)	10–90	91	70–89
AP duration (ms)	0.8–2.4	2.4	0.7–2.8
AHP amplitude (mV)	1.8–5.5	11.9	6–12
dV/dtmax (V/s)	63–226	182	115–291

In conclusion, these studies on nociceptor anatomy and physiology strongly support the hypothesis that the rainbow trout is capable of nociception: the detection of tissue-damaging and potentially painful stimuli.

Brain structures

Fish have the necessary brain areas for nociceptive processing to occur (Figure 4.2; e.g. pons, medulla, thalamus) however, as discussed, the level of differentiation and development in the cortex is crucial in terms of pain perception. Although the cortex becomes less differentiated as we descend the evolutionary tree of vertebrates, fish do possess a rudimentary cortex area. In most fish this takes the form of cerebral hemispheres. In lampreys and hagfishes, the pallium forms the roof of the hemispheres and the subpallium the floor. The striatum runs over the subpallium that extends into the telencephalon medium (Bone 1963, Nieuwenhuys 1977). In the elasmobranchs, the cerebral hemispheres are characterised by large well-defined cell groups with a well-developed thalamic input where the pallial centre receives substantial ascending sensory inputs from the visual, trigeminal and auditory systems (Cohen *et al.* 1973, Platt *et al.* 1974, Bullock & Corwin 1979, Northcutt 1981). In teleosts, this rudimentary cortex or the cerebral hemispheres are better developed with the hemispheric zones possessing complex projections to the diencephalon and midbrain (Northcutt 1981). The pallium and subpallium receive neural projections from the thalamus (Ito *et al.* 1986) and regions of the pallium have been shown to be involved in the processing of somatosensory (e.g. possibly nociception via the trigeminal nerve) communication (Ito *et al.* 1986, Chandroo *et al.* 2004). Tracing studies in the zebrafish, *Danio rerio*, identified afferent nuclei to the pallium: the olfactory bulb, dorsal entopeduncular, parvocellular; preoptic and suprachiasmatic nuclei; anterior, dorsal and central posterior dorsal thalamic, rostrolateral nuclei; periventricular nucleus of the posterior tuberculum; posterior tuberal

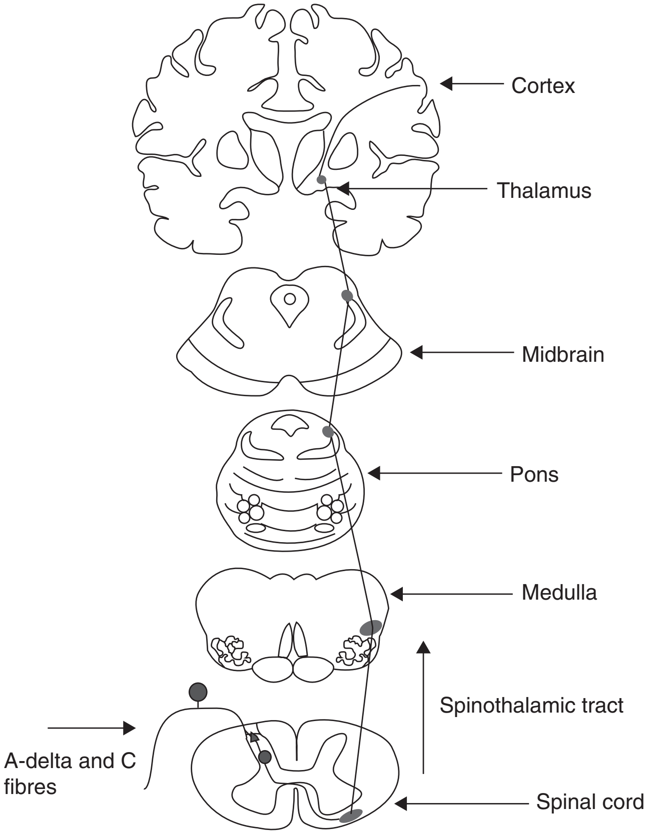

Figure 4.2 A diagrammatic representation of the spinothalamic tract in humans. Nociceptive information in mammals is conducted via C and A-delta fibres from the periphery of the body to the spinal cord via the dorsal horn and terminate in the substantia gelatinosa. The information then crosses the spinal cord to the spinothalamic tract and ascends through the pons, medulla and thalamus to the cortex.

nucleus; tuberal hypothalamic nuclei; dorsal tegmental nucleus; superior reticular nucleus; locus coeruleus; and the superior raphe nucleus (Figure 4.3; Rink & Wullimann 2004). Efferent projections terminate in other telencephalic areas, and also in the habenula periventricular pretectum, paracommisural nucleus, posterior dorsal thalamus, preoptic region, midline posterior tuberculum, tuberal hypothalamus and interpeduncular nucleus. The thalamus is essential for pain processing in humans (Figure 4.2) and ascending information is conveyed to the cortex where descending information is relayed back to the thalamus. These connections between the cortical regions and the thalamus also exist in *D. rerio* (Rink & Wullimann 2004) but have yet to be studied with regard to nociceptive processing.

Recently, electrophysiological recordings have been taken from the spine, cerebellum, tectum and telencephalon of rainbow trout, and goldfish, *Carassius auratus*, during nociceptive stimulation (a pin prod) and innocuous stimulation (brush application, Dunlop & Laming 2004). Neuronal activity was recorded in response to both

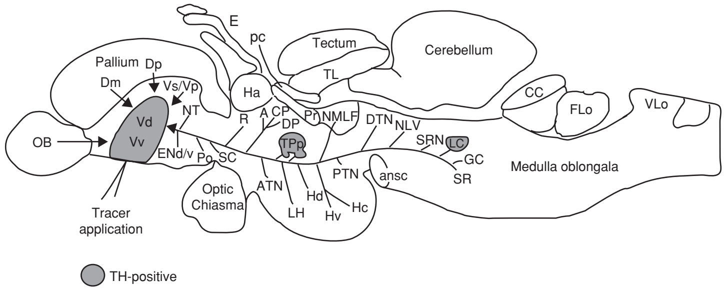

Figure 4.3 Lateral view of zebrafish brain showing all inputs to subpallium (Vd/Vv) demonstrating the complex connections between cortical regions and the rest of the fish brain. This illustrates that cortical connections between the pallium and the thalamus that are essential for pain processing in humans do exist in the fish. Black area: tracer application site; gray shaded areas: neurons double-labelled for tyrosine hydroxylase and retrograde neuronal tracing from ventral telencephalic area. Selected abbreviations for figure, as referred to in text above: A, anterior thalamic nucleus; CP, central posterior thalamic nucleus; Dm, medial zone of dorsal telencephalic area; Dp, posterior zone of dorsal telencephalic area; DP, dorsal posterior thalamic nucleus; DTN, dorsal tegmental nucleus; ENd/v, dorsal part of entopeduncular nucleus; Ha, habenula; OB, olfactory bulb; PTN, posterior tuberal nucleus; SC, suprachiasmatic nucleus; SRN, superior reticular nucleus; TPp, periventricular nucleus of posterior tuberculum; Vd, dorsal nucleus of ventral telencephalic area; VLo, ventrolateral thalamic nucleus. (Reprinted from Rink & Wulliman (2004) *Brain Research*, **1011**, 206–20, with kind permission from Elsevier.)

stimulus types in all these brain areas and the characteristics of the responses differed according to the stimulus type. Importantly, this activity in the telencephalon during peripheral stimulation suggests a nociceptive pathway from the periphery to the higher brain. One of the main aims for the future must be to elucidate the role of these brain areas in the processing of nociceptive signals. Preliminary studies comparing gene expression of controls and fish that had received nociceptive stimulation have shown differential gene expression in the hindbrain, midbrain and forebrain (Reilly *et al*. 2004).

Nociceptive information is relayed to the brain from the periphery via two major tracts. The trigeminal tract conveys information from the head, while the spinothalamic tract conveys information from the rest of the body. Similar to the trigeminal tract in mammals, the mesencephalic nucleus and ascending projections to the thalamus as well as the spinal or descending nucleus of the trigeminal have been found in carp, *Cyprinus carpio* (Luiten 1975) and sturgeon, *Acipenser oxyrhynchus* (New & Northcutt 1984). These nuclei are present in an elasmobranch, the dogfish, *Scyliorhinus canicula* (Rodríguez-Moldes *et al.* 1993, Anadón *et al.* 2000), however, they are not present in the agnathans: the lampreys and hagfish (Northcutt 1979, Ronan 1988), suggesting that the nuclei evolved between the agnathans and the emergence of the fish groups. In the carp, *C. carpio*, the trigeminal clearly projects to the thalamus as it does in other vertebrates, therefore, the trigeminal tract exists in the fishes. Both elasmobranch (Ebbesson & Hodde 1981) and teleost (Goehler & Finger 1996, Finger 2000) groups have the same basic components of ascending spinal projections as higher vertebrates. Finger (2000) stated that the

similarities in connectivity between the teleost sea robin, *Prionotus carolinus*, and other vertebrates are striking. While fish do possess a spinothalamic and trigeminal tract that is comparable to higher vertebrates, studies on how nociceptive information is conveyed by these tracts to higher brain areas are lacking in fish.

Opioids and endogenous substances

Research on the mechanisms by which noxious, potentially painful stimuli are transduced and processed in higher vertebrates have benefited from a number of powerful molecular and cellular methodologies over recent years (Lewin *et al.* 2004). Although a rich variety of molecules have been shown to play a role in pain behaviour and pathophysiological pain in higher vertebrates, these molecules have not been investigated with regards to nociception transduction in fish. However, many studies have examined the presence and distribution of several of these key molecules.

The possession of opioid receptors, endogenous opioids and enkephalins is one of the requirements to determine whether nociception can occur in an animal (Bateson 1991). The mammalian nociceptive system is known to have a mechanism that decreases its own sensitivity. Endogenous opioids and enkephalins bind to opioid receptors/enkephalin synapse receptors on interneurons in the pain pathways in the central nervous system to provide an intrinsic pain suppressing system (Zieglgänsberger 1986). Opioids elicit antinociception or analgesia through three distinct types of receptors, designated as μ, d and k in mammals (Newman *et al.* 2000) and these three receptors have been identified in the zebrafish, *D. rerio* (Stevens 2004).

Opiate receptors and enkephalin-like substances have also been found in various brain areas of goldfish, *C. auratus* (Schulman *et al.* 1981, Finger 1982), catfish, *Clarias batrachus* (Finger 1982), African lungfish, *Protopterus annectens* (Reiner & Northcutt 1987) and rainbow trout, *O. mykiss* (Vecino *et al.* 1991). The distribution of enkephalins in the fish brain shows a similar pattern to that seen in higher vertebrates (Simantov *et al.* 1977, Vecino *et al.* 1992), being found in the telencephalon, nucleus ventromedialis of the thalamus, nucleus lateralis tuberis, nucleus recessus lateralis and nucleus recessus posterioris. Enkephalin-like activity has also been seen in the mesencephalic tegmentum, medial torus semicircularis and the cerebellum. The highest density of enkephalin-like stained fibres were found in the telencephalon in the area ventralis telencephalic, the mesencephalic tegmentum and the dorsal horn of the spinal cord of rainbow trout (Vecino *et al.* 1992). Enkephalin-like activity shows a similar pattern in the Atlantic salmon, *Salmo salar* (Vecino *et al.* 1995). In the spinal cord, enkephalin-like immunoreactivity is most dense in the superficial portion of lamina A, which is thought to be similar to the substantia gelatinosa of mammals (Snow *et al.* 1996). In elasmobranchs, both Met-enkephalin and Leu-enkephalin have been identified but dynorphin-related peptides appear to be absent (Vallarino *et al.* 1994). These two enkephalins are found in distinct nuclei

of the dogfish, *S. canicula*, brain and in particular the pallium of the telencephalon. The distribution is similar to that found in higher vertebrates.

The RFamide-related peptide family are believed to be endogenous opioid agonists and are involved in nociception (Kanetoh *et al.* 2003). In the catfish *C. batrachus*, there is a close relationship with FMRF-related peptides (FMRF: Phe-Met-Arg-Phe-NH_2peptide) and the central opiate system with morphine causing transport or release of the FMRFamide peptides (Khan *et al.* 1998). These peptides are also found in similar brain areas in trout and higher vertebrates (Castro *et al.* 2001). The actions of morphine can also be blocked by the antagonists naloxone and MIF-1 (Ehrensing *et al.* 1982). Therefore, a comparable distribution of opiate receptors and substances is found in both elasmobranchs and teleosts with the fundamental physiological mechanisms similar to that seen in higher animals.

Other molecules involved in pain in higher vertebrates also provide evidence for nociceptive processes similar to those of higher vertebrates. For example, the distribution of immunoreactivity to serotonin, substance P, somatostatin, calcitonin gene related peptide (CGRP), neuropeptide Y and bombesinin in a variety of elasmobranch species is strikingly similar to mammals (Ebbesson & Hodde 1981, Cameron *et al.* 1990).

In vivo observations

A simple reflex response to a noxious stimulus indicates nociceptive function, however, adverse effects on an animal's normal behaviour beyond a simple reflex may indicate a psychological component that is indicative of suffering, and suggests that the animal is perceiving pain. Although subjective, measuring an animal's behavioural reaction to a noxious stimulus is one of the best methods available for assessing pain in animals (Bateson 1991).

A recent study investigated the behavioural response of rainbow trout which had been given subcutaneous injections of acetic acid and bee venom (algesics) to the lips (Sneddon *et al.* 2003a). These fish showed an enhanced respiration rate for approximately 3 hours, did not feed within this period, and showed anomalous behaviours such as rubbing of the affected area on the aquarium substrate and glass and rocking from side to side on either pectoral fin (Sneddon 2003b, Sneddon *et al.* 2003a). Handled controls and saline injected fish did not show these behaviours, did not show such a great increase in respiration rate and began feeding around 80 minutes after treatment. The noxiously stimulated fish only resumed feeding once the behavioural and physiological affects of the bee venom and acetic acid had subsided (approximately 180 minutes). These, therefore, appear to represent changes in behaviour over a prolonged period of time as a result of nociceptive stimulation. Similarly, increases in respiration have also been exhibited by higher vertebrates that experienced a painful event (Kato *et al.* 2001).

The ability of analgesics to modulate nociceptive responses is also indicative of pain perception. The adverse behavioural responses seen in the rainbow trout were quantified and when morphine was administered to fish injected with acid, there

was a dramatic reduction in this rubbing behaviour as well as rocking behaviour and the enhanced respiration rate was also ameliorated (Sneddon 2003b, Sneddon *et al.* 2003a). Therefore, morphine appears to reduce nociceptive responses in this teleost fish. Opiate-induced changes in behavioural reaction to nociceptive stimuli have also been shown in goldfish, *C. auratus*. Ehrensing *et al.* (1982) assessed behavioural response to an electrical current administered by an electric prod. The voltage was gradually increased until an abrupt twitch over the entire length of the body was observed and the corresponding voltage was taken as that at which pain was perceived by the fish. When morphine (an analgesic) was administered, the voltage required to elicit this response was significantly higher. Beyond this, MIF-1 and naloxone (opiate antagonists) were found to block the effects of morphine, decreasing the voltage required to elicit a response. These antagonists have shown similar effects on morphine action in mammals (Ehrensing *et al.* 1982).

Learnt avoidance studies have done much to improve our knowledge of pain and associated fear in animals and this is discussed in full below. Studies have shown that goldfish are able to learn to avoid noxious, potentially painful stimuli such as electric shock (Portavella *et al.* 2002, 2004). Fish can be conditioned to associate a noxious stimulus with a neutral stimulus such as a coloured light, and the fish subsequently perform avoidance behaviour in response to this neutral stimulus alone. Learnt avoidance of a stimulus associated with a noxious experience has also been observed in other fish species (Overmier & Hollis 1983, 1990) including common carp, *Cyprinus carpio*, and pike, *Esox lucius*, avoiding hooks in angling trials (Beukema 1970a, b).

In summary, fish, specifically teleost fish since most studies have examined this group, do appear to fulfil the criteria for nociception and there is the real potential for pain perception. Studies have confirmed that agnathans and teleosts possess nociceptors and the physiological and anatomical properties of these nociceptors are similar to those seen in higher vertebrates. The fish groups do possess the brain areas and pathways leading to higher brain centres that are necessary for nociception to occur in other vertebrate models. The key molecules involved in nociception in mammals are present in fish and are generally distributed in similar areas of the brain and spinal cord to those seen in mammals and humans. Analgesics have been shown to reduce the behavioural response to nociceptive stimuli and learned avoidance to noxious stimuli has been demonstrated in those fish species tested. In two teleost species, profound physiological and behavioural changes are apparent whilst the fish endure a noxious event and these responses were shown over a prolonged period of time rather than instantaneous reflex responses. This indirect evidence would suggest pain perception rather than a nociceptive reflex.

Fear

Like nociception and pain, fear serves a function that is fundamental to survival. Fear is the activation of a defensive behavioural system that protects animals or

humans against potentially dangerous environmental threats (Fendt & Fanselow 1999). In higher vertebrates, these behavioural mechanisms are often accompanied by a range of autonomic changes (LeDoux 2000), such as increased heart rate (Black & deToledo 1972), endogenous analgesia (Bolles & Fanselow 1980), and the release of several hormones such as cortisol (Tomie *et al.* 2002). In humans, these responses are correlated with the subjective state of fear (Bradley *et al.* 1993, Jones 1997). Therefore, when investigating fear in animals it is these behavioural and physiological responses that are measured. Three main criteria can be used to assess the validity of animal (largely rodent) models of human pain (Fendt & Fanselow 1999) and as such these criteria may also provide insight when considering fear in other animals. (1) The systems that control fear response should be similar (have a common neuronal basis) to those neural systems that mediate human fear and anxiety. (2) A variety of clearly threatening stimuli should generate a consistent set of behaviours that protect the individual against the threat. (3) Drugs that modulate human fear and anxiety should show similar effects in the animal. These criteria will be considered in turn to assess the potential of fear in fish.

Neuronal control mechanisms

While some fear responses are innate, others can be learned. Studies on avoidance behaviour have done much to improve our understanding of the neuronal basis and mechanisms involved with fear (LeDoux 2000). Innate or unlearned fear response experiments use stimuli that naturally provoke fear without the animal having previous experience of the stimuli (e.g. natural predators, Utne-Palm 2001; or pheromone alarm substances, Hall & Suboski 1995). Learned fear response studies use repeated pairings of an initially neutral stimulus (the conditioned stimulus, CS) with an aversive event (e.g. electric shock, the unconditioned stimulus, US). As the relation between the two stimuli is learned, the behavioural and physiological responses to the US come under the control of the CS (LeDoux 2000). After a few CS–US pairings the CS elicits the fear response. This Pavlovian conditioning has been observed throughout the phyla (e.g. in flies, worms, fish, birds, and mammals).

In higher vertebrates, fear conditioning is thought to be an integral part of defensive behaviour and involves mainly the amygdaloid and hippocampal regions of the brain although other areas are also implicated. Studies in fish have shown that these responses also appear to be dependent upon cognitive mechanisms and homologous limbic brain regions in the telencephalon. In mammals, the amygdala has long been known to be important in arousal and emotions, particularly fear response (Carter 1996, Maren 2001). The dorsomedial (Dm) telecephalon in fish has been implicated in emotional learning and is thought to be homologous to the amygdala in mammals (Bradford 1995, Butler 2000, Portavella *et al.* 2004). In higher vertebrates, the hippocampus is involved in memory and learning of spatial relationships and it is the dorsolateral (Dl) telencephalon in fish that is thought to be functionally homologous to the hippocampus.

Lesion or electrical stimulation of the Dm telencephalon has been shown to affect aggressive behaviour in fish (Marino-Neto & Sabbatini 1983, Bradford 1995) and ablation of the telencephalon of fish produces deficits in spatial and associative learning (Salas *et al.* 1996a, b, Lopez *et al.* 2000a, b). Through similar lesioning studies, Portavella *et al.* (2002, 2004) have studied the specific contribution of the Dm and Dl telencephalon to different types of learning and memory. Results from this work suggest that the Dm telencephalon may be involved in emotional learning and the Dl telencephalon may be involved in spatial or temporal learning. Similar studies in mammals have shown that lesions to the amygdala impair emotional conditioning (Lorenzini *et al.* 1991, Aggleton 1992, Eichenbaum *et al.* 1992, Killcross *et al.* 1997) while lesions to the hippocampus produce deficits in spatial and associative learning (Olton *et al.* 1987, Eichenbaum *et al.* 1992, Kesner 1998, Portavella *et al.* 2002). Therefore, Dm and Dl areas of the fish telencephalon share functional similarities with the amygdala and hippocampus respectively, in mammals.

Learning is thought to be mediated in part by receptors in the brain that are activated by N-methyl-D-aspartic acid (NMDA). Administration of selective antagonists of NMDA receptors impair learning mechanisms such as associative learning and conditioned fear in mammals (Miserendino *et al.* 1990, Kim *et al.* 1991, Sanger & Joly 1991, Maren 2001). Experiments with goldfish have shown that intracranial administration of MK-801, an NMDA receptor antagonist, blocks specific aspects of Pavlovian fear conditioning in fish (Xu & Davis 1992, Davis & Klinger 1994, Xu *et al.* 1995, Xu 1997) in similar ways to that seen in rats (Xu 1997).

Together, the above indicates a common neuronal basis for the fear response control systems of fish and higher vertebrates.

Consistent behavioural and physiological responses

Learned response experiments also enable experimenters to assess and measure consistent behavioural and physiological responses to fearful stimuli. Studies on fear conditioning in mammals often measure levels of freezing and startle behaviour (Fendt & Fanselow 1999). In fish, a number of different behavioural responses to potentially threatening stimuli have been described and include escape responses such as fast starts (Domenici & Blake 1997, Chandroo *et al.* 2004, Yue *et al.* 2004) or erratic movement (Cantalupo *et al.* 1995, Bisazza *et al.* 1998), as well as freezing and sinking in the water (Berejikian *et al.* 1999, 2003). Such behaviours may serve to protect the individual from the threat and a number of studies have illustrated that these behaviours can be shown in response to an US through learned association with a CS.

Many fish species release chemical alarm substances when injured. These are thought to act as warning signals, as conspecifics show an innate behavioural fright response to these chemicals (Smith 1992, Lebedeva *et al.* 1994, Brown & Smith 1997, Berejikian *et al.* 1999). For example, Crucian carp, *Carassius carassius*, exposed to skin extract from conspecifics killed by decapitation showed

significantly reduced feeding behaviour and increased alarm behaviours when compared to fish exposed to a control substance (physiological saline) and the neutral amino acid L-alanine (Hamdani *et al*. 2000). These alarm behaviours included dashing movements, vigorous movements in the aquarium substrate, and fast swimming towards hiding places, remaining there for an extended period. These behaviours are thought to be associated with predator evasion (Hamdani *et al*. 2000). Pearl dace, *Semotilus margarita*, show a clear behavioural response to alarm pheromone and a simultaneous increase in plasma concentrations of cortisol and glucose, indicating a stress response (Rehnberg *et al*. 1987).

Berejikian *et al*. (1999, 2003) have shown that chinook salmon *Oncorhynchus tshawytscha*, show an innate anti-predator response to the odour of a potential predator (the northern pikeminnow, *Ptychocheilus oregonensis*) which involved higher levels of freezing behaviour and a reduction in feeding levels when compared to fish exposed to control substances. This response was enhanced when the predator odour was presented in combination with skin muscle extract (containing alarm substance) taken from chinook salmon killed by a single blow. Here, the response to the two odours involved less time feeding, more time motionless and more time in the lower part of the aquarium when compared to those exposed to the predator odour alone. These responses were also significantly greater than those seen in fish that were presented with the predator odour in combination with skin muscle extract from another species (the green swordtail, *Xiphophorus helleri*). Pairing a predator odour with a conspecific alarm substance in this way has also been shown to condition the enhanced behavioural fright response to a predator odour alone (e.g. Magurran 1989, Chivers & Smith 1994, Chivers *et al.* 1995, Brown & Smith 1998). Chinook salmon exposed to the combination of conspecific alarm substance and predator odour do appear to acquire predator recognition, as these fish showed more time motionless in response to the subsequent exposure of predator odour alone when compared to those fish that had previously been exposed to the swordtail extract and predator odour (Berejikian *et al.* 1999, 2003).

Learned avoidance studies not only show that a consistent suite of behaviours are produced in response to fearful stimuli, they also provide evidence that the displayed behaviour is not merely a reflex response. Learning to avoid an aversive stimulus in the future implies a cognitive process of recognising that the behavioural response will lead to the desired effect of avoidance (Yue *et al*. 2004).

Mosquitofish, *Girardinus falcatus*, perform a fast start escape response to a simulated predator (Cantalupo *et al.* 1995). In this experiment, the predator was only presented when the fish swam across the median portion of the tank. Interestingly, with repeated exposure the fish began to avoid this particular portion of the tank. If the fish happened to swim in the tank centre it tended to perform an erratic zig-zag movement (Bisazza *et al*. 1998, Chandroo *et al*. 2004, Yue *et al*. 2004). It has been suggested that this apparent learned association of the predator threat with a particular spatial area may require involvement of brain areas homologous to the hippocampus, as the spatial aspects of fearful stimuli are processed by the

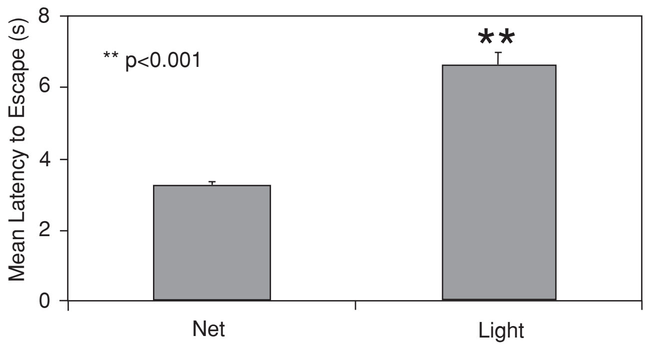

Figure 4.4 Latency times of fish showing an escape response to a plunging net and after the fish had learned to associate the light stimulus with the plunging net. The data show that the fish took approximately double the time to respond to the light (CS) when compared to the net (US) and it has been proposed that this is indicative of a more conscious voluntary response to the former. (Reprinted from Yue *et al*. (2004) *Applied Animal Behaviour Science*, **87**, 343–54, with kind permission from Elsevier.)

hippocampus and transmitted to the amygdala in associative learning in higher vertebrates (Maren 2001, Chandroo *et al.* 2004). The avoidance or zig-zag escape strategy prevented the predator stimulation or removed the fish from the previously perceived negative stimulus and therefore it has been suggested that the fish could anticipate this frightening stimulus and the escape response was in part motivated by an affective state of fear (Chandroo *et al*. 2004, Yue *et al*. 2004).

In a recent study, rainbow trout learned to avoid an automated plunging net by rapidly swimming (shuttling) through a door to a separate section of the experimental tank (Yue *et al.* 2004). A CS, a 10-second light signal prior to the beginning of the net drop, was then paired to the plunging net (US) and the fish subsequently learned to associate the light with the net stimulus, showing the shuttle behaviour in response to the light signal alone. Interestingly, the avoidance behaviour to the light alone differed from that shown following the net plunge. The latency of the fish's response to the stimulus (defined as the time between the onset of the stimulus and the moment the fish completely passed through the doorway) was significantly higher for the light alone (approximately 6 seconds) when compared to the net (approximately 3 seconds) (Figure 4.4). The authors suggest that this indicates the involvement of a mental process and that the fish were demonstrating a more conscious voluntary response to the light alone when compared to the reflexive fast start response to the net (Yue *et al*. 2004) although this is confounded by the fact that a conditioning paradigm was used. This may support the suggestion that an affective state such as fear may serve to motivate behaviour in fish.

Recently it has been shown that physiological responses in fish can also be elicited by a CS following learned association with an US (Moreira & Volpato 2004). Here, a 1-minute light signal (CS) was given immediately before a grid was lowered into the aquarium, confining the fish (Nile tilapia, *Oreochromis niloticus*) to a small area (US) for 30 minutes. This was repeated once a day for 9 days. On the

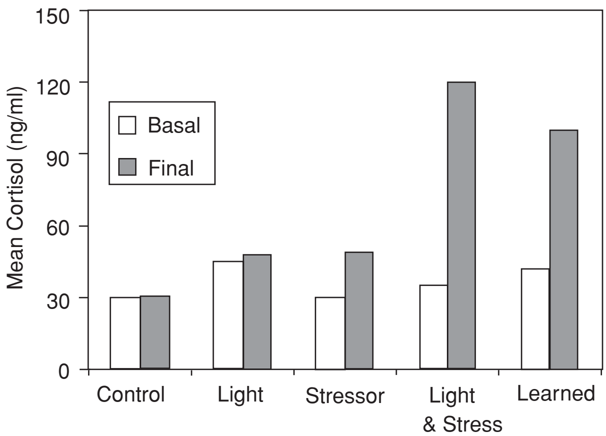

Figure 4.5 Mean plasma cortisol levels in Nile tilapia prior to (basal) and after (final) five different 10-day procedures. Cortisol levels indicate that the fish in the learning treatment group showed a conditioned cortisol response to the light stimulus alone that was similar to that seen in fish that experienced the light and the US stressor. This cortisol response was significantly higher than basal levels recorded in the same fish. The other treatment groups indicate that neither the light alone nor the procedure carried out over the previous 9 days were sufficient to induce a cortisol increase. (Reprinted from Moreira & Volpato (2004) *Journal of Fish Biology*, 64, 961–9, with kind permission from Blackwell Publishing.)

tenth day the fish showed significantly increased cortisol levels in response to the light alone when compared to baseline measures prior to conditioning (Figure 4.5). These cortisol levels were similar to those shown by a control group that received the same protocol but were subjected to the CS–US pairing again on this tenth day. The fish that showed this conditioned cortisol response to the light also showed higher cortisol levels than control groups of fish that had not been manipulated, fish that had been subjected to the light signals in the absence of the US, and fish that had received the US for the previous 9 days. Therefore, the conditioned fish appear to have shown a stress response in association with a non-stressful CS through learned association with a stressful event. It may be that this rise in cortisol levels was in anticipation of this stressful event to enable the fish to cope with the stressful event. Similar conditioned corticosterone responses have been observed in rats and humans (Buske-Kirschbaum *et al.* 1996, Sabbioni *et al.* 1997, Cordero *et al.* 1998, Stockhorst *et al.* 2000, Tomie *et al.* 2002).

Effects of anti-anxiety drugs

Much of the work on developing anxiety-reducing drugs for humans has been carried out on rodents. This work has described the actions of benzodiazepines, such as diazepam, at specific sites in the brain and related these actions to changes in behaviour. However, there has been far less research into these mechanisms and anxiety-like states in other species. Binding sites for benzodiazepines have been found in the brains of fishes in the same areas that are found in mammals (Nielsen

et al. 1978, Hebebrand *et al.* 1988, Rehnberg *et al.* 1989) and administration of the benzodiazepine drug chlordiazepoxide has been shown to reduce attacking in agonistic encounters between male Siamese fighting fish, *B. splendens*, without inducing noticeable toxic or sedative effects (Figler *et al.* 1975). Studies on fathead minnows, *Pimephales promelas*, treated with chlordiazepoxide provide evidence to suggest that the benzodiazepine receptors in the central nervous system of fish may serve to modify behaviour in anxiety-like states in similar ways to those seen in higher vertebrates (Rehnberg *et al.* 1989). When exposed to an alarm substance, the control minnows displayed a behavioural alarm response and when a chemical feeding stimulus was released into the water while the fish were still in an alarmed state these fish showed low levels of exploratory behaviour. However, when fish were exposed to high, but non-sedative, levels of chlordiazepoxide they did not show the fright response to the alarm substance and displayed vigorous exploration when presented with the chemical feeding stimulus (Rehnberg *et al.* 1989). Rodents that have been administered benzodiazepines show similar changes in exploratory behaviour (Crawley 1985, Rehnberg *et al.* 1989).

To conclude, when assessing the motivational state of fear, researchers have looked to the behavioural and physiological changes that accompany fear in humans. Specifically, the existence of a common neuronal mechanism that controls fear response in higher vertebrates, consistent behavioural and physiological responses to a variety of clearly threatening stimuli, and the ability of anti-anxiety drugs to reduce fear response can be used to gauge an animal's capacity to experience fear. Based on these criteria it would seem that fish have the potential to experience a fear-like state.

Conclusions

If we accept that fish are capable of experiencing some form of pain and fear then we have to accept that their well-being is impaired when we subject them to any tissue damaging or fear-causing event. Humane methods for animal slaughter are based on the principle that the animal is killed quickly with minimum fear and pain. However, aquaculture and recreational angling slaughter methods have been developed not to minimise stress but to achieve product quality control, efficiency and processor safety (Conte 2004). Methods vary and include electrical stunning followed by decapitation, blunt trauma to the cranium, and percussive stunning using a captive bolt. A variety of injuries, stress reactions, and mortalities occur during capture and release of fish by hook and line (Chopin & Arimoto 1995) and the use of a variety of nets in both commercial (Thompson *et al.* 1971) and recreational fishing (Steeger *et al.* 1994, Pottinger 1997, Cooke & Hogle 2000, Barthel *et al.* 2003) can also cause abrasion injuries leaving open lesions that are readily infected with bacteria and fungi (Barthel *et al.* 2003). Damage also occurs when fish collide with commercial trawling nets or gear, evident in injuries to jaws

and vertebral column (Miyashita *et al.* 2000), and skin injuries are sustained when escaping from trawl cod ends and through trawl nets (Suuronen *et al.* 1996, Olla *et al.* 1997). Aggressive behaviour is a well-known problem in aquaculture where injuries to dorsal, pectoral and caudal fins, eyes and opercula are common and can have a range of effects on feeding behaviour and growth through to infections and even mortality (Abbott & Dill 1985, Turnbull 1992, Turnbull *et al.* 1998, Greaves & Tuene 2001). Many finfish are subject to tagging procedures, in particular fin clipping as well as other invasive methods, and it is not known if these are painful although tagging does elicit a physiological stress response (Sharpe *et al.* 1998).

Although approached separately in this chapter, pain and fear are by no means exclusive of one another. This is well illustrated by a recent study using rainbow trout. When compared to controls, noxiously stimulated trout did not show an appropriate fear response to a fear-causing stimulus (Sneddon *et al.* 2003b). Theories on attention propose that there is a limited capacity or pool of attention and that pain may take priority, or 'soak up', a large amount of this pool, leaving little capacity for competing stimuli (Kuhajda *et al.* 2002). This would suggest that the consequences of nociception may involve higher processing. In the trout, the noxious experience may have dominated attention and therefore normal responses to the fearful stimulus were not seen. Little work has been conducted on other species but common carp do show similar anomalous behaviours whilst enduring acetic acid injection as given to the trout (Reilly *et al.* 2004). Many clinical studies have shown that humans do not perform as well on other tasks when in pain (Kuhajda *et al.* 2002). A reduction in the normal fear response in any animal would serve to reduce the functions of that response, namely threat or predator avoidance. Theoretically there is, therefore, the possibility that a noxious stimulus may be indirectly damaging to a fish by reducing predator vigilance. This remains to be tested, however Ryer (2002) has shown that walleye pollock, *Theragra chalcogramma*, exposed to a simulated trawl-stressor were less likely to avoid a predator and were consumed in greater numbers by a predator when compared to controls.

It is clear that we must take an ethical approach to the welfare of fish and since there is significant evidence to suggest that their well-being is adversely affected by potentially painful and fearful situations, it is our moral responsibility to reduce any possible suffering and discomfort. A plethora of studies are now being conducted that are aimed at improving our equipment and methods to make procedures less invasive for the fish and improve welfare. In theory, if we improve well-being, productivity is enhanced and a better economic return can be made, therefore it is in our interests to consider fish welfare issues.

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Chapter 5

Can Fish Suffer?

Victoria A. Braithwaite and Philip Boulcott

Introduction

Humans increasingly interact with fish: they are farmed and fished for our consumption; they are used in biological and medical research; they are kept as pets; and they provide an opportunity for sport. Given these levels of association it would seem timely to reflect on the influences our relationships have on the well-being of fish. However, determining what fish require for good welfare, or defining when fish welfare has been compromised, is not an easy task. Recent evidence has confirmed that fish have specialised pain receptors, nociceptors, and our increasing knowledge about the cognitive abilities of fish are beginning to provide us with a framework to assess the capacity for fish pain perception and suffering. Yet this is only one side of the debate, with other work questioning whether fish are capable of suffering or experiencing pain (see Rose 2002). The objective of this review is to outline a range of studies and observations that attempt to answer many of the issues raised in this debate.

Animal welfare as a field has made considerable progress over the last two decades (e.g. Dawkins 1998, Mendl 2001, Sørensen *et al.* 2001). To date, however, most of the work has focused on terrestrial animals, and relatively little work has addressed the welfare requirements of fish. Until recently, fish were generally considered to be animals that were unable to perceive pain, and had little or no memory. As such they have tended to fall through the welfare net. There is, however, growing evidence that teleost fish are far from dim-witted (Huntingford 2003), and that they share many of the same general pain processes and much of the same stress physiology as other vertebrates (Barton & Iwama 1991, Sneddon *et al.* 2003a).

It has been known for some time that fish do not respond well to prolonged periods of stress (see Chapter 3); chronic exposure to aversive conditions can generate changes in the immune system that make fish more vulnerable to disease (Pickering & Pottinger 1989). Work such as this has led to the conclusion that fish stress physiology has many parallels with the stress processes that occur in terrestrial vertebrates (Wedemeyer *et al.* 1990, Barton & Iwama 1991, Wendelaar Bonga 1997). Moreover, recent research with rainbow trout (*Oncorhynchus mykiss*) by Sneddon *et al.* (2003a) indicates that fish are capable of detecting noxious or

potentially painful stimuli by way of cutaneous nociceptors (specialised receptors that respond to noxious stimuli) located around the head. When these receptors are stimulated, fish exhibit physiological responses and perform aversive behaviours similar to those observed when terrestrial vertebrates react to noxious stimulation (Sneddon *et al.* 2003a, b, see Chapter 4). Despite this evidence, however, it is difficult to determine whether fish have an awareness of stress and pain, i.e. whether they have the capacity to experience pain and suffering.

Current interest in issues relating to welfare in fish has resulted in the publication of a number of documents indicating that there should be concern about the potential pain, stress and suffering that may be caused to fish when they interact with humans. For example, the Fisheries Society of the British Isles published a briefing document on fish welfare in 2002 (http://www.fsbi.org.uk/docs/brief-welfare-refs.pdf). The Care and Use of Fish in Research, Teaching and Testing published by the Canadian Council on Animal Care in 2005 also puts forward a treatise on similar lines (discussed by Griffin & Gauthier 2003). These documents and a few recently published reviews indicate that there should be concern about fish welfare (Braithwaite & Huntingford 2004, Chandroo *et al.* 2004a). This, however, is not a universally held belief and one author in particular has questioned the evidence that fish can experience pain (Rose 2002). Rose argues that fish brains are not sufficiently developed and lack key structures required for conscious perception of pain in humans. He argues that human psychological experience of pain requires the presence of a highly developed neocortex (the outermost, convoluted part of the mammalian forebrain) and in particular, the frontal lobe cortex, which is thought to play an important role in this process. Rose therefore concludes that conscious perception of suffering is unique to humans and higher primates because appropriately developed neocortical structures are only found in these animals. This is an extreme point of view and is not a line of argument that is supported by many who work on animal pain (e.g. Broom 1991, Bateson 1992, Gentle 1992, Molony *et al.* 2002). Rose does acknowledge that fish can generate neuroendocrine and physiological stress responses to noxious stimuli, but he suggests that these are entirely non-conscious reactions. Similarly, he concludes that fish should be housed appropriately and handled in ways that will not induce physiological stress. He does not, however, consider that human interactions with fish cause them to suffer.

Rose summarises a large body of work in his 2002 review and he makes a compelling argument, nevertheless, there are many examples where he only reports evidence that supports his viewpoint despite there being other studies suggesting alternative interpretations. Thus, it is timely to reflect on the issues concerning welfare in fish and to consider whether the dismissal of the capacity for fish pain and suffering is justified.

This chapter begins with an overview of the brain organisation in a typical teleost and then discusses how fish brains, despite being relatively simple in comparison with other vertebrates, possess several regions that are homologous to regions found in terrestrial vertebrate brains. The emergence of a significant body of work over

the last decade is then also assessed which demonstrates that fish are capable of remarkably sophisticated behaviour indicative of complex cognitive processing. It is concluded that there is no easy way to definitively answer the question, ‘Can fish suffer?’, however, considering the current literature, it is argued that they should certainly be given the benefit of the doubt.

Basic organisation of the teleost fish brain

Early work on the brain and behaviour of teleosts indicated that their behaviour was predominantly governed by the motor systems in the brainstem. Forebrain structures were described as small and poorly developed, serving only to modulate brainstem-generated behaviour (Overmier & Hollis 1983, Overmier & Papini 1986). However, this over-simplified description fails to recognise the diversity found in fish brains. For example, it does not acknowledge that certain species have specialised senses with more developed sensory processing regions than others (see Kotraschal *et al.* 1998). Current understanding is changing quickly, and over the last 15 years our knowledge of the teleost brain has grown considerably (Broglio *et al.* 2003). Comparative studies and lesion work have revealed not just complexity in the fish brain, but also homology between specific regions in teleost and terrestrial vertebrate brains (Kotraschal *et al.* 1998, Portavella *et al.* 2002, Broglio *et al.* 2003).

Teleost brains share many of the same classical subdivisions seen in most vertebrates: a brain stem; the hindbrain; the mesencephalon (midbrain); a cerebellum; a pair of optic lobes; the paired cerebral hemispheres of the telencephalon (forebrain); and the associated olfactory bulbs (Figure 5.1). In fish, somatosensory

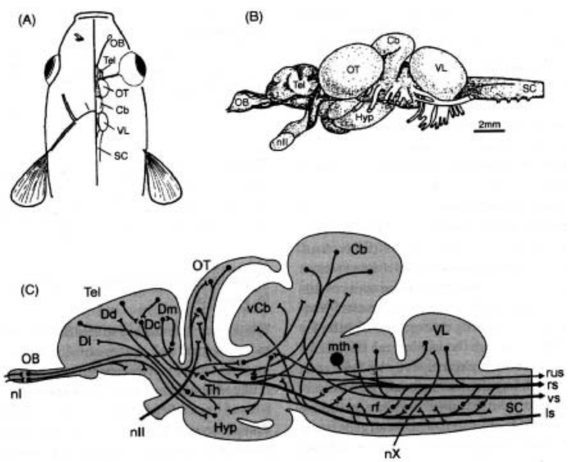

Figure 5.1 Lateral view of the goldfish brain showing the main macroanatomical structures. Cb, cerebellum; Hyp, hypothalamic lobe; nII, optic nerve; OB, olfactory bulb; OT, optic tectum; SC, spinal cord; Tel, telencephalon; VL, vagal lobe (adapted from Broglio *et al.* 2003).

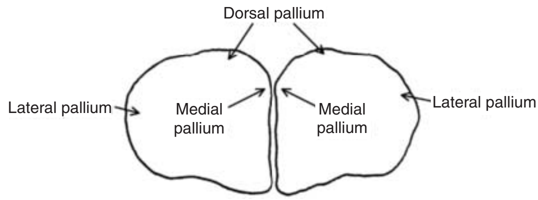

Figure 5.2 Schematic of a coronal section of a goldfish telencephalon (forebrain) with different substructures labelled. The top of the figure represents the front of the brain.

information reaches the brain primarily through specialised cranial nerves, in particular the trigeminus, facialis, vagus and three lateral line nerves. The brain stem and the tegmentum of the mesencephalon and diencephalon (the region of the midbrain containing the thalamus and the hypothalamus) are continuous with each other. Various sensory inputs can be integrated in the paired inferior lobes of the hypothalamus and these are important structures because they can influence fish behaviour through the secretion of hormones. The cerebellum, which serves a range of functions, varies in size across different species of teleost (Finger 1983) and is particularly large in electrosensitive fish, such as the ghost knifefish, *Apteronotus leptorhynchus* (Maler *et al.* 1991). The paired telencephalon hemispheres of the forebrain arise from the rostral section of the neural tube, with the olfactory bulbs arising from the rostral tip. Large projection neurons connect the telencephalon and the diencephalon via the olfactory tracts. Thus, overall, there is considerable interconnectivity between different areas of the teleost brain, some of which can be seen in Figure 5.1c.

Considering the forebrain in a little more detail, there are subdivisions between the pallial areas towards the roof of the forebrain, and subpallial areas underneath (Figure 5.2). The pallial region can be further subdivided into central and peripheral regions, where the distinction between these areas is attributed to the central region having large scattered neurons. The peripheral area is composed of three regions: the medial, dorsal and lateral pallial nuclei (Macphail 1982). Research has recently found that the dorsal and lateral areas of the pallial nuclei are homologous with the amygdala and the hippocampus found in avian and mammalian brains (Portavella *et al.* 2004).

The forebrain receives sensory information from a variety of sources: visual cues, olfactory cues and lateral line information picked up by mechanoreceptors and electroreceptors (Finger 1980). There is remarkable similarity between the lateral line organisation in fish and the auditory systems found in terrestrial vertebrates, both relying on mechanosensory receptors that utilise hair cells to pick up information. The forebrain plays a key role in processing and coordinating sensory and motor information. However, as Macphail (1982) discusses, there also appears to be sufficient capacity for a number of other higher-level processes to occur within this structure. Indeed, a common theme of many recent studies examining the teleost brain is the key role the forebrain plays in influencing behaviour.

Are teleost brains complex enough to allow fish to suffer?

Regrettably, in his dismissal of pain and suffering in fish, Rose (2002) reviews a rather select series of studies indicating that learning in fish is basic, and he concludes that it is restricted to associative learning which requires no form of conscious awareness, that is, fish learn by forming associations between stimuli – in the same way that Pavlov's dogs learned to salivate when a bell rang because the bell always preceded food. Thus Rose concludes that fish behaviour is limited, highly stereotyped and invariant within a species. The crux of this stance is that fish do not have a capacity for complex cognitive processing or for the emotional experiences of pain and suffering because they have simple brains that lack a neocortex prohibiting them from conscious awareness. There are several lines of argument that counter this conclusion. First, it is not clear that the neocortex is a unique structure that provides an animal with a capacity for self-awareness, or consciousness (see Chandroo *et al.* 2004b). For example, some authors suggest that processes required for consciousness occur in more centrally located structures such as the thalamocortical system (Edelman & Tononi 2000, Laureys *et al*. 2000). Also, consciousness is proposed to arise through the integration and relaying of multiple pieces of information between divergent areas of the brain (Tononi & Edelman 1998, Baars 2002). Second, a wider survey of the current literature on fish cognition clearly demonstrates that several fish species are capable of learning and integrating multiple pieces of information that require more complex processes than associative learning (Braithwaite 2006). For example, fish can learn to combine different types of spatial information, such as landmarks and the geometrical layout of an area to create a map-like representation that allows them to plan routes (Sovrano *et al.* 2003, Vargas *et al.* 2004). Some species, for example, guppies (*Poecilia reticulata*) have also been shown to be capable of learning and remembering individuals in their school (Griffiths 2003). Third, there are several examples where different taxonomic groups have neural specialisations that allow them to process information in different ways. For example, comparisons of avian and mammalian visual systems clearly demonstrate how different animals can perceive and process the same type of information but through different pathways and neural structures. In birds, ascending visual information primarily passes through the optic tectum, but in mammals it passes through the lateral geniculate nucleus (Shimizu & Karten 1993). Similarly, Marino (2002) reports how the forebrains of cetaceans and primates are organised in very different ways, yet there are considerable similarities in various aspects of their cognitive abilities, and these persist despite the differences in the forebrain architecture. Extrapolating from this, the ability to suffer and perceive an emotional experience associated with pain may not be solely restricted to the prefrontal cortex of humans and higher primates; other animals may process similar information in different ways. Rather than relying on comparative examples such as those used by Rose (2002), it would seem more appropriate to identify taxon-specific capacities for pain perception and suffering.

Do fish alter their behaviour in response to noxious stimuli?

Fish are known to change their behaviour as a consequence of aversive stimulation (Portavella *et al.* 2002, Sneddon *et al.* 2003a, b, Yue *et al.* 2004). Responses to aversive stimuli can include freezing (a period of watchful immobility) or stimulus-initiated startle and may also result in a cessation of normal activities such as feeding (Godin 1997). However, assessing the 'motivational affected state' of fish – a concept that encompasses a wide range of conscious experiences such as pain, fear, hunger, thirst or pleasure (Chandroo *et al.* 2004b) – owing to its subjectivity, presents a difficult task to the researcher (Paul *et al.* 2005). Such states are biological adaptations that underpin particular behaviours (Chandroo *et al.* 2004b), producing a strong motivation for a particular behaviour in those situations where a flexible or learned response is more adaptive than a rigid or reflexive one (Fraser & Duncan 1998). Hence, while studies in fish welfare benefit greatly from techniques previously used in human pain studies – such as the examination of responses in heart rate, respiratory rate and muscle physiology – a clear picture of the subjective state of an animal can only be obtained indirectly from evidence gained from behavioural observations.

One such study employing the concept of motivational affective states in tandem with physiological responses has been able to provide evidence for the perception of pain in fish. Rainbow trout treated with noxious chemical stimuli (e.g. acetic acid or bee venom) showed a prolonged decreased motivation to feed and a dramatically increased opercula beat rate in comparison to identically handled saline treated control fish (Sneddon *et al.* 2003a). In an extension of these investigations, attentional state was investigated in fish after they were given a noxious stimulus. Trout usually try to avoid novel objects, which some have interpreted as a 'fear' response. Avoidance can therefore be quantified by the amount of time a fish spends avoiding a novel object temporarily placed into its home tank. Sneddon *et al*. (2003b) observed that the avoidance response was reduced in fish that had acetic acid injected into the skin around the mouth. These results suggest that when fish respond to nociceptive stimulation they are distracted, paying less attention to a novel object and so spend more time closer to it. This hypothesis is supported by the fact that the administration of a pain relieving analgesic (morphine) largely restores the fear response (Sneddon *et al.* 2003b). These results are similar to earlier work by Ehrensing *et al*. (1982) who found that intracranial application of morphine in goldfish (*Carassius auratus*) decreased their response to electric shock. When opiate antagonists were then applied, the response to the electric shock resumed. One problem associated with the approach used by Ehrensing *et al*. (1982) is that the application of morphine might be expected to reduce reactions to a stimulus regardless of whether it was associated with pain or not. The novel object avoidance experiment of Sneddon *et al*. (2003b) overcomes this criticism, however, because in their study the effect of the morphine revived novel object avoidance behaviour seen in the fish.

Whilst other research has attempted to examine motivational affective states in fish (e.g. Yue *et al*. 2004, Dunlop *et al*. 2006), empirical evidence suggesting that fish have the capacity for fear and suffering is only just emerging. To make such inferences, experiments must be able to differentiate between behaviour driven by the animal's affective state and that simply governed by associative learning. The motivations underpinning this behaviour are, of course, subjective (Dawkins 1993, Duncan 2002), and in order to determine that fear is involved, it will be necessary to address whether the aversion response under examination equates to what may reasonably be considered as a negative affected state (Paul *et al*. 2005).

Perhaps one of the most elegant studies investigating the potential complexity of the neural response to aversive stimuli in fish comes from a group of experimental psychologists based in Seville, Spain. Through a series of experiments combining brain lesions with behavioural observations, they have highlighted the complex role that several areas of the brain play in controlling different types of learning and memory task in goldfish. Portavella *et al*. (2002) trained goldfish to avoid an area of a tank using the simultaneous presentation of a green light and an electric shock. Fish were then given different types of brain lesion surgery and their ability to perform the avoidance in response to the green light was measured. A lesion to the dorsomedial area of the forebrain (Figure 5.2) impaired the avoidance response. However, fish given a sham (control) lesion, or a lesion to the dorsolateral forebrain showed no impairment in their performance (Figures 5.2 and 5.3a). In a second study, fish were given the light cue 5 seconds before a shock was given. In this type of task both avoidance and timing information is important. When the different lesions were carried out, fish with either dorsomedial or dorsolateral lesions were impaired at performing the avoidance response, but the sham (control) fish showed no deficit in their performance (Figure 5.3b). Finally, goldfish were trained in a spatial task and then lesioned in the same ways as before. In this instance, only fish with dorsolateral lesions showed impaired performance (Figure 5.3c). Taken together, these three experiments indicate that the dorsolateral and dorsomedial regions of the goldfish brain process different types of information. Thus, the anticipated aversive stimuli generate avoidance behaviour, but not through simple learned associative responses. Rather, they involve the integration of different types of information processed in different parts of the brain.

Furthermore, the dorsomedial forebrain region investigated by Portavella *et al.* is linked to learning and memory of affected motivated behaviours (Davis *et al.* 1976, Paul *et al.* 2005); the simultaneous presentation of light and shock affect the emotional learning and memory aspects of the task. Thus, their data supports the homology between the dorsomedial region of the teleost brain and the amygdala of the mammalian brain, an area known to be tightly linked to emotion (Killross *et al.* 1997). Aspects of the task that require timing or spatial relationships to be encoded are processed in the dorsolateral areas of the forebrain (Portavella *et al.* 2002) and appear to be homologous with the mammalian hippocampus, a specialised area that

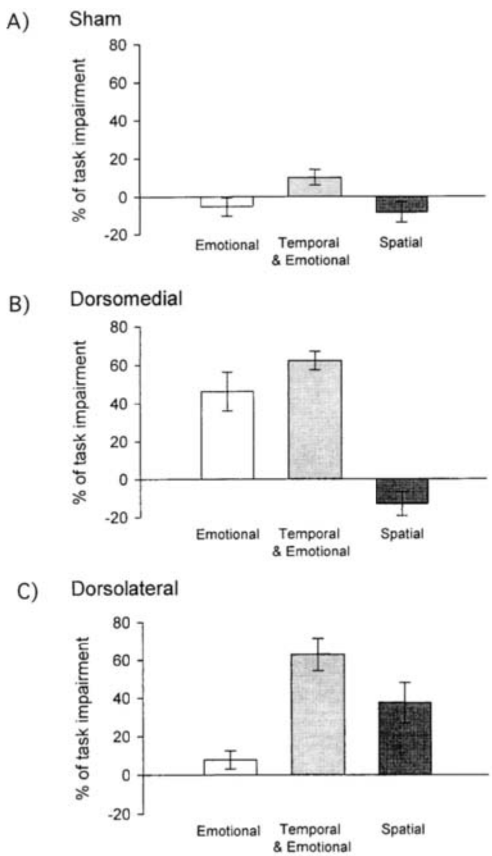

Figure 5.3 Learning impairments observed after different types of lesion to the brain of a goldfish: (a) sham (control lesion); (b) lesion to the dorsomedial (Dm) part of the telencephalon; (c) lesion to the dorsolateral (Dl) part of the telencephalon. Bars represent standard errors. *Emotional learning:* mean proportion of avoidance responses observed after lesioning. In this task the fish were conditioned to actively avoid a stimulus when the conditioned stimulus (CS) and the unconditioned stimulus (US) overlapped. *Temporal and emotional learning:* mean proportion of avoidance responses observed after lesioning. In this task the fish were trained to actively avoid a stimulus but here there was an inter-stimulus gap between CS and US. *Spatial learning:* mean proportion of reversal task impairment in a spatial maze. (Based on Portavella *et al.* 2002.)

processes information on the timing of events and information relating to orientation or spatial behaviour (Eichenbaum *et al.* 1992).

The question of whether the emotional response of fish to aversive stimuli is comparable to humans may not be essential when assessing welfare needs. Instead, the use of motivational affective states is becoming more prevalent in the evaluation of animal welfare in general (Broom 1998). Thus it becomes necessary to show that fish have the behavioural and physiological attributes that characterise conscious cognition or motivational affected states (Paul *et al.* 2005). Approaches such as those taken by Portavella *et al.* (2002) are certainly laying the foundations for these

types of method. The successful application of such approaches will necessarily require the classifications of behaviours associated with pain and fear in fish as they have in other species (Duncan 2002). Nevertheless, many of the responses fish have to aversive stimuli are similar to those found in other vertebrates and, given that fish brains have the capacity to remember, such findings provide evidence for the potential for fish to maintain an awareness of pain and may suggest some form of capacity for long-term suffering.

Cognitive capacities in fish – a capacity for long-term suffering?

Critiques on animal welfare often state that fish do not have the brain capacity for complex cognitive processing, a claim echoed in the work of Rose (2002). If true, it could be argued that such capacities restrict suffering to the short-term and will lessen any welfare issues associated with fish well-being. However, in contrast to this view, it is now becoming increasingly accepted that fish are capable of relatively complex behaviours governed by a range of cognitive processes (Laland *et al.* 2003, Braithwaite 2006). In this final section, to illustrate these points, a few well-worked examples are highlighted which show the types of complex learning and memory processes that fish are capable of.

There are several examples of fish learning to recognise specific individuals or groups of individuals. Some species recognise and remember individuals that they encounter, and this can result in decreased aggression when these individuals come across each other again (Johnsson 1997, Utne-Palm & Hart 2000). In other cases, schooling fish such as guppies can learn to recognise multiple members of a school (Griffiths & Magurran 1997), and species such as the European minnow (*Phoxinus phoxinus*) can learn to discriminate the competitive abilities of different schools and use this information to decide which school to join (Metcalfe & Thomson 1995). Presumably the advantage of discriminating between good and poor competitors is that a fish feeding on a limited food supply has a better chance of securing more food if it is foraging with poor competitors. Male Siamese fighting fish (*Betta splendens*) can also watch and assess aggressive interactions between neighbouring fish (Oliveira *et al.* 1998). Information on the relative fighting ability of neighbours is then used in decisions about future aggressive interactions between neighbours and observers. It has also been shown that threat displays given by the males vary depending on the presence or absence of female conspecifics (Doutrelant *et al.* 2001). For example, in the presence of an observing female, males reduce the amount of aggression within their display, by decreasing behaviour such as biting. The responses that the fish are making in these different examples require multiple pieces of information to be integrated and processed before the fish can perform an appropriate behaviour.

There are also many examples where fish learn by watching others; this is sometimes referred to as social learning (Heyes 1994). It has a number of advantages

over trial and error learning as an individual that learns to copy others can avoid making costly mistakes itself, and social learning has been shown to influence a range of fish behaviours from foraging to mate choice (Brown & Laland 2003). For example, fish can observe knowledgeable demonstrators in order to learn a new route through a simple maze (Laland & Williams 1997). Coolen *et al.* (2003) have also demonstrated that social learning can influence foraging decisions. For instance, nine-spined sticklebacks (*Pungitius pungitius*) often hide among weed and watch other fish as they are foraging, and then use this socially learned information to guide their own foraging behaviour. Interestingly, they can socially learn about different patch profitabilities not only from conspecific fish, but also from another species, the three-spined stickleback (*Gasterosteus aculeatus*).

A particular area in which fish cognition has been exceptionally productive has been in studies involved in spatial learning and memory. There are now several examples illustrating that fish can move from one point to another by taking the most efficient route, or they learn to move around their environment in ways that minimise the chance of encountering a predator (see Odling-Smee & Braithwaite 2003). The fish learn and use a number of cues to help them orientate around their environment. Some learn to make a series of body-centred, or egocentric, movements or turns (Rodríguez *et al.* 1994, Girvan & Braithwaite 1998). In other cases, fish can find their way around by keeping track of their own movement relative to one or more external reference points, and sometimes these may be used to form a map (Rodríguez *et al.* 1994, Sovrano *et al.* 2003). These strategies provide fish with an ability to take shortcuts or select between alternative routes to reach a goal without having to rely on a specific sequence of locations or landmarks.

Being able to generate mental representations is considered by some to demonstrate 'primary consciousness' (for review see Chandroo *et al.* 2004a), in other words, a capacity to integrate multiple pieces of information and use these to generate a mental representation. Perhaps one of the most impressive illustrations of the use of an internal map comes from the work of Aronson (1951, 1971) who observed the escape responses of rock pool gobiid fish (*Bathygobius soporator*). Aronson discovered that fish learn the topography of the rock pool area at high tide, such that when they are trapped in one rock pool at low tide they can accurately jump and escape into an alternative pool when threatened. However, only fish that have been exposed to the rock pool area at high tide are successful in finding rock pools for their escape. This impressive feat of spatial learning is rapid as well: the gobies required only one exposure at high tide to accurately learn a potential escape route (Aronson 1971).

Conclusions

The examples given in this chapter highlight that many fish can perform complex behaviours, and that these are generated even though teleosts have a relatively simple

brain and nervous system (Kotraschal *et al.* 1998). Despite their simplicity, teleost fish represent the most abundant and diverse group of vertebrates that have evolved and have radiated into a wide range of niches (it has been estimated that there are over 30 000 species of teleost, Kullander 1999). In association with this divergence, fish brains, their nervous systems and, in particular, their sensory systems have also diverged and become specialised (Kotraschal *et al.* 1998). Furthermore, it is now clear that several features found in the terrestrial vertebrate brain have an evolutionary history that can be traced back to the fish brain (Broglio *et al.* 2003). The homologies that are now being described have been both striking and surprising.

In summary, the continuing body of work focusing on fish pain and suffering has produced a picture of an animal which is capable of responding physiologically to pain, and whose normal suite of behavioural repertoires alters in response to aversive stimuli, sometimes over long periods of time. Despite their relatively simple nervous systems, teleosts are capable of complex and flexible behaviours, are capable of learning and remembering complex information, and with these capabilities comes the potential for long-term suffering. However, equating the experiences of fish to aversive stimuli to what humans experience as emotion will only lead to scientific error. When considering the welfare of these fish it will, therefore, be necessary to develop techniques that examine whether or not fish are in a negative or positive affected state, and whether this has implications for its welfare. This is an emerging field and much work needs to be done on species that are most commonly used for our needs. That fish are the most diverse of the vertebrate groups will have implications also, as responses to certain conditions are unlikely to be universal, and this will have ramifications for welfare guidelines and legislation established for their protection.

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Chapter 6

Welfare Legislation Applying to Farmed Fish in the UK

Andrew Voas

Introduction

Current animal welfare legislation applies to fish in certain circumstances and covers farming, killing and transport. This article summarises the main provisions of existing and proposed legislation in UK and also mentions other approaches that the government may take to improve fish welfare without involving legislation.

The Protection of Animals Act 1911

The Protection of Animals (Scotland) Act 1912

The Protection of Animals Act 1911 consolidated various pieces of legislation concerning cruelty to animals from the nineteenth century and applies to England and Wales. The Protection of Animals (Scotland) Act 1912 contains equivalent provisions for Scotland. Section 1.1a of these Acts makes it an offence to: 'wantonly or unreasonably do or omit to do any act causing unnecessary suffering to any animal; or cause, procure, or, being the owner, commit any such act'. The Acts apply to any domestic or captive animal and legal advice received by the Scottish Executive for the Environment and Rural Affairs (SEERAD) has been that they would therefore apply to ornamental and farmed fish, including those kept in sea cages.

The Acts introduced the concept of 'unnecessary suffering' which can be reviewed depending on developments in current knowledge. It is probably fair to say that when the Acts were written they were not intended to apply to fish but as our understanding of the capacity of fish to suffer has developed, so courts will be able to interpret the phrase 'unnecessary suffering' in the light of existing knowledge. This therefore remains a potentially useful piece of legislation despite dating from almost 100 years ago.

To prove an offence under these Acts would require evidence of suffering, and that this was unnecessary and unreasonable. Although no prosecutions concerning

fish farming have been initiated, an example of where the Acts could apply might be if sea cages were located in an unsuitable position where the nets could be deformed by currents or storms. If this was the case, and it could be shown that fish had suffered as a result of unreasonably siting the nets, then an offence under the relevant Act might have been committed.

The Agriculture (Miscellaneous Provisions) Act 1968

This Act applies to England, Wales and Scotland. Fish are not specifically included or excluded in this legislation, but it applies to any creature kept for the production of food. Section 1.1 states that: 'any person who causes unnecessary pain or unnecessary distress to any livestock for the time being situated on agricultural land and under his control or permit any such livestock to suffer any such pain or distress of which he knows or may reasonably be expected to know shall be guilty of an offence'. Legal advice has been that 'agricultural land' would include fish kept for the purpose of food production in fresh water or in saltwater tanks on land, but would not apply to fish kept in sea cages.

This legislation followed the Brambell Committee Report (Brambell 1965) and concern about factory farming in the 1960s. The wording of the legislation was a slight advance on the 1911 and 1912 Acts as it became an offence to cause unnecessary pain or unnecessary distress without this having to be demonstrated to be 'unreasonable'. It also provides for welfare codes for farmed livestock and for rights of access to land for inspectors to determine if an offence may have been committed.

The Welfare of Farmed Animals Regulations 2000

The Welfare of Farmed Animals (Scotland) Regulations 2000

Under these Regulations, owners and keepers of animals kept for farming purposes must 'ensure the welfare of animals under their care' and 'ensure that the animals are not caused any unnecessary pain, suffering or injury'. These Regulations specifically include fish in their scope but they are made under the Agriculture (Miscellaneous Provisions) Act 1968 so again are limited to animals kept on 'agricultural land' which does not include fish kept in sea cages.

These Regulations arise from our obligation to implement European legislation, in this case Council Directive 98/58/EC concerning the protection of animals kept for farming purposes. The general obligation to ensure the welfare of animals kept for farming purposes shows a change in emphasis from the 1911/12 and 1968 Acts which made it an offence to cause unnecessary suffering, to now also placing an obligation on the keeper to positively promote the welfare of the animal. These

Regulations are useful in assisting enforcement action in that they allow for the service of improvement notices in situations where an inspector thinks that animals may be caused suffering if certain improvements are not made.

The Welfare of Animals (Slaughter or Killing) Regulations 1995

These Regulations, which cover England, Wales and Scotland, apply to animals bred and kept for the production of meat, skin, fur, or other products so can be taken to apply to farmed fish. Regulation 4.1 requires that: 'no person engaged in the movement, lairaging, restraint, stunning, slaughter and killing of animals shall cause any avoidable excitement, pain or suffering to any animal; or permit any animal to sustain any avoidable excitement, pain or suffering'. Regulation 4.2 requires that: 'no person shall engage in the movement, lairaging, restraint, stunning, slaughter or killing of any animal unless he has the knowledge and skill necessary to perform those tasks humanely and efficiently . . . '. There is therefore a general requirement that anyone killing farmed fish should have received sufficient training to kill fish humanely without causing unnecessary pain. Although there is a system for the licensing of slaughtermen who kill terrestrial animals in abattoirs and which is administered by the Meat Hygiene Service, there is no equivalent requirement to licence individuals involved in killing fish.

The Welfare of Animals (Transport) (Scotland) Regulations 2006

The Welfare of Animals (Transport) (England) Order 2006

The Welfare of Animals (Transport) (Wales) Order 2007

This legislation replaced The Welfare of Animals (Transport) Order 1997 to implement Council Regulation (EC) 1/2005 and requires that animals are transported in a way that does not, and is not likely to, cause injury or unnecessary suffering. It applies to all commercial transportation of animals including fish, and there are some specific provisions for the transport of fish including the need to transport them in suitable containers and with sufficient liquid. An example of where this order could apply to farmed fish might be if fish are being transported in a way that causes injury. If, for example, a problem is identified in a suction system for moving fish from one site to another, and it is known that the system is likely to cause injury, then it could be an offence under the Order to continue to use the system.

New welfare acts

New legislation on animal welfare is being introduced in England, Wales and Scotland. The Animal Health and Welfare (Scotland) Act 2006 and the Animal Welfare Act 2007 came into force in October 2006 and April 2007 respectively. These Acts will consolidate, update and replace various parts of existing welfare legislation including the 1911/12 and 1968 Acts. They continue the change in approach over the last century from preventing cruelty to also promoting welfare, as it will become the duty of any animal keeper to ensure the welfare of the animal and to follow codes of practice concerning welfare where these exist.

These welfare Acts introduce a duty of care to all vertebrates kept by man. They clearly cover all ornamental and farmed fish, including farmed fish kept in sea cages, so removing the anomaly of the Agriculture (Miscellaneous Provisions) Act 1968 which only applies to fish held on agricultural land. The Acts have a very wide scope, covering the keeping of pets as well as farmed animals and they are primarily intended as enabling legislation to allow further detailed legislation on specific areas of concern to be introduced at a later date. However, the legislation will not apply to anything done in the normal course of commercial or recreational fishing or angling.

Influences on legislation

The original animal welfare legislation from the nineteenth century and early twentieth century was introduced in response to public concern about particular issues, often represented via lobby groups, and public opinion remains a significant influence on legislation today. However, later in the twentieth century scientific research was increasingly used to provide evidence when considering changes in the legislation, and the Farm Animal Welfare Council (FAWC) was established to provide independent scientific advice to government on welfare issues.

After joining the European Union, British welfare legislation has been introduced to implement EC Directives agreed by member states. These often follow consideration of issues by the Council of Europe. In future, the Office International des Epizooties (OIE) is likely to play an increasing role in setting recognised animal welfare standards internationally, in the same way that it sets recognised standards for the diagnosis and control of animal disease at present.

Enforcement of welfare legislation

There is a difference between having welfare legislation in place and being able to enforce it in practice, as the State Veterinary Service and other enforcement bodies understand from work involving terrestrial farm animals. Successful prosecutions require good evidence and must be initiated within six months of the offence

occurring. This may seem a reasonable period, but it is often surprisingly difficult in complex cases to gather statements and other evidence to present to the relevant prosecuting body in England and Wales, or to the Procurator Fiscal who decides whether to proceed with a case in Scotland.

The subsequent legal process is equally time consuming and it will normally take several months between an offence being committed and a trial. During this period there may be ongoing welfare problems on farms which need to be addressed, and this can be a difficult situation for the enforcement bodies to deal with. Where cases do reach court, it can sometimes be frustrating for those who have been involved in preparing a case to find that it may fail due to what may seem to them to be a legal technicality, or when a conviction is secured, it can sometimes seem to them that the sentence does not fully reflect the seriousness of the offence.

For these reasons, prosecutions tend to be used as a last resort reserved for persistent offenders and more serious cases, and in most other cases an advisory approach is taken when welfare problems are found on farm.

Other approaches to improving animal welfare

It should be recognised that legislation sets a basic minimum standard for animal welfare, but producing legislation is not all that is done by the government to promote welfare. Government supports a significant amount of research into animal welfare, including fish welfare and this may bring about improvements in welfare directly through improved knowledge within the industry, or be used as a sound basis when developing future legislation.

A good example of this has been the research on electrical trout stunning which arose following concerns expressed in the 1996 Farm Animal Welfare Council Report on the Welfare of Farmed Fish (FAWC 1996). This led to a project as part of the government's LINK collaborative research programme with industry, the Humane Slaughter Association and government funding, to find an effective way of killing trout using electricity rather than allowing them to suffocate in air as was standard practice at harvesting. Although the initial equipment had practical difficulties, the technology has now been developed into a reasonably practical device and there is a firm commitment from the industry to introduce this method within a short period.

The government also promotes the development of quality assurance schemes, which include a welfare component, and the development of welfare codes for different species. For terrestrial farm animals, detailed welfare codes have been developed in consultation with the industry. These are statutory codes in that, although contravening the code in itself is not an offence, contravention of the code may be used as evidence to support a prosecution if animals are caused unnecessary pain or distress as a result.

In Scotland, the need for a voluntary welfare code for fish farming was recognised in the Strategic Framework for Scottish Aquaculture in 2003, which gave a

commitment to producing a welfare code by 2004. It was realised that discussions in the Council of Europe on recommendations for fish welfare had been taking place over several years and the code would draw on some of the outcome of these discussions. The code was developed by the Scottish Aquaculture Health Joint Working Group, which includes representatives of SEERAD, the industry, the Scottish Society for the Prevention of Cruelty to Animals (SSPCA), Compassion in World Farming (CIWF), State Veterinary Service (SVS) and the Responsible Use of Medicines in Agriculture Alliance (RUMA). It was an important principle from the start that recommendations were to be based on available scientific evidence, and that this would be a working document to be updated in the light of future scientific evidence. The Code of Good Practice for Scottish Finfish Aquaculture has now been produced and is available at http://www.scotland.gov.uk/resource/doc/1062/0004654.pdf.

The Code accepts the five freedoms principle used by the Farm Animal Welfare Council as far as these can be applied to fish, that is: freedom from hunger and malnutrition; freedom from discomfort; freedom from pain, injury and disease; freedom to express normal behaviour and freedom from fear and distress. The Code covers veterinary health plans, stockmanship, water quality, stocking density, grading, transport, disease, harvest and euthanasia. It is recognised that water quality parameters are crucial to fish welfare and where water quality parameters have been agreed by the industry on the basis of scientific evidence, these have been given and will be updated as new scientific evidence becomes available.

Conclusions

Over the last century, welfare legislation has progressed from dealing with cruelty after it has occurred to also requiring the promotion of welfare of all vertebrates kept by man. This has reflected developing concern and scientific evidence about the capacity of animals to suffer and how they can benefit from improvements to the way in which they are kept. In future, legislation will clearly consider the welfare of all farmed fish and be applicable to them. Codes of practice will also become increasingly important, not only to ensure compliance with minimum legal requirements but also to demonstrate best practice which will be demanded by industry quality assurance schemes.

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Part II
Aquaculture and Fish Welfare

Chapter 7
Fish Farmer's Perspective of Welfare

Nick Read

Introduction

As consumers have become more reliant on major retailers for the supply of their daily bread and thus progressively more remote from the source of their food, a counter-wave of interest has been building as to the conditions under which that food is produced. This has long since focused attention on the welfare of fish on fish farms, which have tended to be open to public inspection and interest as the industry has developed.

So, what is the farmer's perspective on this? Where does fish welfare feature in the minds and in the daily work of fish farmers? The simple answer is that fish welfare is central to the success and even to the survival of fish farming businesses. Much of the media attention may be irritating to farmers to the extent that it is often ill-informed and unrealistic, but it is fully accepted that what happens on the farms is vital to fish welfare.

Fish welfare is discussed at different levels by stakeholders in the industry. As an example, academics debate whether fish feel pain, and the degree and nature of that feeling. Fish farmers listen to that debate with interest but have long since accepted, as a working hypothesis, that their fish can feel pain and that the way they operate their farms should reflect this. This attitude is driven by a combination of emotion, ethics and financial interest. It is generally assumed by fish farmers that a fit and healthy fish will grow better and will, ultimately, result in a better financial return. It is assumed that a fit and healthy fish is enjoying good welfare and that any deterioration in welfare rapidly shows itself in fish health problems. Any discussion as to whether a fish farmer might offer reduced welfare in the pursuit of greater financial reward appears to the fish farmer to be ridiculous. The challenge facing the farmer is to ensure that good health and fitness is achieved.

For many years, the range of species farmed in UK was restricted to salmonids with minor activity in coarse fish production. The scene is now fast changing with cod, turbot, halibut, sea bass, bream, Dover sole, tilapia and barramundi already added to the list and, no doubt, with more to come. Each of these species will carry its own welfare challenges when farmed, but aquaculture as a science has developed

fast over recent decades and fish farmers are now much better placed to understand these challenges and to respond to them.

The species that have been longest established in fish farming in the west are trout species and they can be used as an example to explain the types of challenge that arise. Much of what applies in trout will also arise in the farming of other species.

Trout farming

Trout farming takes place at a wide variety of sites offering site specific opportunities and constraints, so it is worth reviewing briefly the size and scale of the industry before going on to look at the on-farm factors that influence welfare and how the fish farmer is involved.

The farming of trout has been taking place in the UK since the late 1800s but expanded to become a food producing industry only when pelleted feeds were developed in the 1960s. These feeds offered an immediate and major welfare improvement over the rather crude diets previously available which had lacked the necessary balance of nutrients. The UK trout industry in 2007 comprises around 350 farms, producing approximately 16 000 tonnes of trout a year. Of this production, around 12 000 tonnes is destined for table production and the remainder is grown for restocking fisheries.

Farm sites range in output from under 10 tonnes per year to over 1000 tonnes with a trend towards fewer and larger farms, as the industry matures and rationalises. The majority of farms are owned and operated as family units, although there is a clear move towards the establishment of groups of farm sites coming under the management of one controlling farm management business. This is a response to continuing downward pressure on ex-farm prices which is a long-term feature of all food producing sectors. The size of farm considered viable for table trout production moves relentlessly upward as economics dictate greater production per man-year.

Most trout produced for the table are rainbow trout (*Oncorhynchus mykiss*), with small niche sectors growing golden trout and blue trout. (Golden and blue trout are also *O. mykiss*, and are achieved by stock selection.) Small quantities of brown trout (*Salmo trutta*) are also grown for the table. Around 2% of production is organic (adhering to the Soil Association Organic Standard), covering both rainbow and brown trout. In the restocking sector, again the rainbow trout predominates, with smaller quantities of native brown trout and very small quantities of brook trout (*Salvelinus fontinalis*).

On-farm welfare interactions

Site selection

Most trout farms in the UK were built in the 1970s. Their continued existence would suggest that the sites have been suitably selected, and are not generally liable to

flooding or drought, but all site selection is a compromise. Trout farming requires water of consistently high quality. The ideal, dreamed about by trout farmers, is for a water source where a copious volume of pure water flows out of the ground at 15°C, all year round.

In practice one has to make do with what nature delivers. Pure water sources tend to have small flows, and large flows come with all the impurities that man and passage through the water catchment have added. Rivers can change from being roaring torrents in winter to resembling a dried up gulch in low flow conditions. Water temperatures can range from well below optimal to a long way above. We have seen, recently, an example of a farm that had existed for many years being inundated in a '100 year flood' and the fish stock swept away and out to sea. With global warming, such inundations may become more frequent.

Control and monitoring of environmental parameters

UK trout farming is based on borrowing water from the water source, be it a spring supply or a river, passing it through the farm and then returning it to the water catchment. This 'flow through' arrangement is referred to, by regulatory authorities, as non-consumptive abstraction and discharge. Whatever words one uses to describe the system, it offers limited scope to control the quality parameters affecting fish welfare. In the case of river supply it is also difficult to isolate the farms from pathogens that may exist in the water catchment.

There has been progressive improvement in this control of water quality over the years as the industry has developed. While the volumes of water used preclude control of water temperature; oxygen concentration in the water, and particularly diurnal fluctuation of oxygen content, can be and is controlled by aeration or oxygenation. Monitoring of oxygen concentrations is common on farms to a degree, in line with the level of intensification existing at the site. Attention on more intensive farms is now turning to the control of other dissolved gases such as carbon dioxide and total ammonia. On sites where pH varies or remains outside the optimal range, this can also be adjusted to maintain stable conditions.

Disease control

This is a 'whole of life' battle. The fish starts out in life with a high health status and the aim of the farmer is to maintain that situation through good biosecurity and, as far as possible, the reduction of stress imposed on the fish, particularly chronic stress. Protection through vaccines is available for only one of the most common trout diseases.

On the other side are the pathogens that may come with the water supply or be introduced to the farm by fish or bird movements. Also potentially significant will be mistakes by fish farmers and general poor biosecurity and bad husbandry

practice that will at the least, increase stress levels within the fish population and predispose the fish succumbing to any disease challenges they face.

Feed management

Use of demand feeding systems can improve welfare by having the feed available to the fish in response to their natural rhythms, rather than the daily work schedule of the fish farmer. This is a win-win situation for the fish farmer, resulting in lower stress and better health for the fish by allowing feeding to satiation without the aggressive activity often seen during hand feeding, a more even oxygen demand and ammonia production through the day, and better food conversion.

Stress control

The main aim of the fish farm manager is to maintain conditions for his fish that minimise stress levels. This requires a growing environment where water quality remains, at all times, as close to optimum as is possible. It also means that activities likely to cause stress, such as grading and moving of fish, are reduced to a minimum, and that this work is performed with the welfare of the fish in mind.

Stocking density can be important in terms of its possible effect on water quality, and the farm manager must be constantly looking ahead to ensure that increasing biomass, or a predicted water temperature rise, do not change water quality parameters to a degree where stress levels rise.

Management skills and training

Trout farms are managed by professional people who either have many years of experience or training through one or other of the college courses that are available. The farm manager has a major influence on the welfare of fish under his control through sensitive programming of the work on the farm and by reducing as far as possible the stress that can arise from that work. It is the farmer who makes the initial diagnosis when diseases occur and it is the farmer who monitors general fish welfare on a day-to-day basis.

Whole of life fish welfare

For farmed fish to enjoy good welfare throughout the growing cycle, it is necessary that all of the multiple influences on welfare touched on above remain under control at all times. While trout have been used as the example, the generality holds good for all farmed species. Fish farmers who succeed in balancing all these factors will not only provide good welfare but also good production figures. This requires an affinity for the fish on the part of the manager and passionate commitment.

Industry commitment to welfare

Almost every walk of life is now surrounded by codes of conduct, codes of practice and quality assurance schemes with attendant inspections and accreditation. Aquaculture, being a food producing industry, is no exception.

- Through the Federation of European Aquaculture Producers (FEAP) there is an overarching Code of Conduct for European Aquaculture. This covers the activities of the full range of species farmed across Europe and deals with welfare alongside food safety and environmental protection.
- The trout industry in the UK has had the British Trout Association Code of Practice since 1992, which was revised in 1995 and again in 2002.
- The trout industry also has its own quality standard: Quality Trout UK. This has separate standards for farms and processors and is accredited by the United Kingdom Accreditation Service (UKAS) to EN 45011 and inspected by the European Food Safety Inspection Service (EFSIS). Almost all table trout being supplied to the multiple retailers is now produced under this standard. (BS EN 45011/BSI EN 45011 are criteria for technical and management competence, assessed against agreed international standards.)
- On a broader front, a voluntary code of good practice for aquaculture in general has been produced in Scotland: A Code of Good Practice for Scottish Fin Fish Aquaculture.

Welfare requirements are threaded through all four of these codes and standards, which have become a prerequisite for supply to major retailers. They demand good fish welfare alongside their other requirements of consistent supply, eating quality and protection of the environment. Consumers in the UK care about welfare and, therefore, so does the supply chain.

Perceived welfare issues

When the Farm Animal Welfare Council (FAWC) turned their attention to aquaculture in 1992, they made known a list of their concerns before ever stepping on to a fish farm. Most of these concerns were carried forward from work they had done on pigs and poultry, and at the head of their list were stocking density and slaughter methods. When the FAWC report was published (FAWC 1996), those same perceived problems were duly listed, some without any scientific backing. That necessary scientific knowledge did not then exist, in what was, and is still, a young industry. It is, however, progressively being developed through research projects supported by industry and government.

Retail consumers of trout also carry similar perceptions of welfare problems in aquaculture and their concerns are likely to be wrapped up in the pejorative phrase 'factory farming'.

The reality

Fish farmers see things differently.

- They think more about water quality than stocking density which, taken in isolation, is largely irrelevant at stocking levels found in the industry. There is, at last, good science to support this stance (see Chapters 8 and 10).
- They think about how improvements to the facilities on their farms would improve welfare. Aquacultural Engineering is a branch of science that has come a long way in recent years and is still advancing in terms of feeding control, grading equipment and, in particular, in control of dissolved gas concentrations.
- They think about stock selection and how it could be improved to provide fish that are resistant to endemic disease challenges and are more stress resistant, or domesticated.
- They think about farm health improvement plans. Leading farms have these in operation as part of their quality systems.
- They think about biosecurity and how to improve it while their farms are connected to a river system.
- They worry about how to control disease with the severely restricted range of medicines now available. They can diagnose disease and know what would cure it, but are often prevented from using the suitable treatment because of legislative barriers.
- They think about the use of all-female and triploid stock, to which some critics of the industry take exception, but are sure that, on balance, their use offers far better welfare than the alternative of mixed-sex stock.

Responsibility for welfare

In UK aquaculture, between 70 and 100 million finfish of various species are grown each year. The goal of perfect fish welfare has yet to be fully achieved. Who should share in the responsibility for improvement?

- The fish farmer obviously should. He accepts responsibility for welfare by engaging in fish farming and has a direct vested interest in continual improvement.
- The trade associations for the industry accept a collective responsibility to encourage improved welfare and to seek ways of enabling it to happen.
- The fish vet accepts a share of responsibility by agreeing to prescribe treatments for the fish.
- Feed suppliers are concerned and offer excellent technical support to improve fish welfare and survival. Fish survival underpins payment for the feed they sell.
- Researchers seek to find solutions for the challenges faced by industry and thereby share in responsibility. They are progressively delivering the 'good science' that will point the way forward.

- Medication suppliers, while eager to assist in dealing with fish health problems, are hampered by the complexity and inordinate cost of licensing new treatments and the need to meet overly cautious environmental standards.
- Legislators have allowed a situation to develop where traditional remedies that provided good disease control have been withdrawn, leaving the welfare of millions of fish prejudiced. They have done this in the name of minimal, sometimes merely notional, improvements in human safety. These legislators should feel a responsibility to remedy that situation.
- The more vociferous of the non-governmental organisations (NGOs) are self-appointed consciences for the industry so they share responsibility.
- Retailers and consumers are all prepared to concern themselves with how fish farmers operate their businesses so they also share responsibility.

With all these sectors actively wishing to be considered stakeholders in the welfare of farmed fish, it must be possible to achieve improvement.

Stakeholder action and achievement

Critics of the industry appear to have a blind spot for the considerable action that has been taken and is on-going to raise standards of farmed fish welfare.

The British Trout Association has supported research and development projects for many years and has had outstanding support from UK government bodies, from the Centre for Environment, Fisheries and Aquaculture Science (Cefas) and the Fisheries Research Services (FRS) and from university research teams.

Awaiting publication is a report on stocking density which should redirect attention from this subject towards the more relevant matter of water quality (see Chapter 8). Projects are in progress on:

- Causes of fin erosion and its implications for welfare (see Chapter 9)
- The relationship between water quality and welfare (see Chapter 10)
- Malformations and mortality in the hatchery
- Effluent treatment leading to improved water quality in recirculation systems

For disease treatment there are currently projects on:

- Whitespot
- Proliferative kidney disease (PKD)
- Rainbow trout fry syndrome (RTFS)

These three diseases, together with enteric redmouth (ERM), are currently the major killers of trout which together cost the industry around £5 m a year. This should be set in the context of an ex-farm value for the whole UK trout production of < £30 m a year.

In the area of legislation:

- The Animal Welfare Act confirms the responsibility of fish farmers towards the welfare of fish stock under the control of the farm (see Chapter 6).
- The Council of Europe spent a number of years developing, with the aquaculture industry, legislation on fish welfare but abandoned work on the project in 2007 when close to completion. Other organisations becoming involved are the World Organisation for Animal Health (OiE) and the European Food Safety Authority (EFSA).
- In January 2007, the Welfare of Animals in Transport Regulation came into force and was transposed into local legislation for each Member State and devolved administration. This Regulation was written for land animals and only covers fish because of the inadvertent inclusion at a late stage of drafting, of the words 'vertebrate animals'. British Trout Association has responded by developing a Best Practice Briefing Note.
- Council Directive 2006/88/EC brings the control of fish health in Europe up to date and reflects the growing range of species used in aquaculture. (It is being transposed into local legislation to take effect from August 2008.)

There is a clear willingness among the stakeholders in aquaculture to work together to achieve beneficial results. This is seen in the work of the Scottish Aquaculture Research Fund (SARF) and Aquaculture Wales.

FAWC highlighted humane harvesting as an area that needed research and improvement. The industry accepted the need for change and with support and encouragement from the Humane Slaughter Association, Defra and most of the major retailers, funds were found for work at Bristol University and Silsoe Research Institute, for the development of an electric stunning system that answers the call to achieve instantaneous insensibility and to maintain that state until the fish are dead (see Chapter 14). This has been a superb example of different stakeholders working together to achieve a common aim and it is a field in which the UK now leads the world.

On stocking density there is now good scientific evidence that happens to support the stance taken by the industry but this work has also opened up the way to establish on-farm welfare indicators that can readily be assessed.

Advances in understanding genetics have provided methods for improving brood stock and making trout better adapted to the challenges they face on fish farms. This work has a long way to run but early indications suggest that there are great advances to be made.

Best practice

Almost every area of fish farming is being developed, and training of farm managers and staff is a continuous process. In the case of rainbow trout, this is being carried

out through Quality Trout UK and the Skretting Fish Disease training courses. Most farms now run under one or other of the software control systems offered by the feed companies and these are continually being refined. The combined effect of these developments is to bring higher professional standards to the industry and to continue to improve best practice.

Challenges

- For the future, competition for the use of finite water resources must be expected.
- The trout industry, and indeed aquaculture as a whole, suffers from a lack of suitable licensed treatments and it can only be hoped that there will be concerted action to improve the situation. The small UK trout industry (100 million fish/year) cannot afford the hoops and hurdles set up by EU bureaucracy.
- There are endemic diseases for which solutions, in the form of treatments and/or management protocols, are urgently needed. There is hope of a vaccine for proliferative kidney disease (PKD) within a few years and that would transform trout production on the rivers where the problem is endemic.
- Improvements in water treatment methods are needed to allow more precise control of water chemistry. Water reuse and recirculation aquaculture systems are being adopted by the UK trout industry and it is important to ensure that the theoretical improvements in environment control, and hence in welfare, that they offer are translated into actual improvements. Their potential disadvantage is that the inherent intensification of such systems could result in things going seriously wrong if not managed properly.

Objectives

To speed up improvement in fish welfare, help and encouragement is needed from the various stakeholders:

- From government, a firm resolve to improve the availability of medicines is required. Gentle interest will not suffice; the welfare of large numbers of fish requires vigorous action and tangible results.
- From fish vets, less dependence on sale of antibiotics and more involvement in fish health improvement plans.
- Practical on-farm welfare indicators that farmers, as well as scientists, can use to assess welfare.
- From researchers and pharmaceutical companies, vaccines to replace antibiotics and a deeper understanding of the organisms that adversely interact with our fish so that farmers can manage their way around them.
- From fish breeders, fish that are resistant to the diseases that are endemic in the areas where we farm and that are better adapted to farming.

- From critics and campaigning organisations, sensible discussion and participation in the solution of issues, as epitomised by the approach taken by the Humane Slaughter Association over trout harvesting methods. That has achieved a real improvement in the way that millions of fish are harvested. If any organisation feels that there is an issue that needs to be dealt with, let them open a dialogue with the industry.

Conclusions

Fish farmers have listened politely to the list of evils supposedly existing on fish farms and find little in the content that reflects the reality of fish farming today, but rather a repetition of the perceived problems of yesteryear.

Fish farmers believe that they can point to an excellent track record of improvement in welfare and a commitment to continuing improvement. They have been proactive in developing real knowledge and understanding of the subject and good science is starting to flow across an area that was dominated by prejudice, ignorance and superstition.

Given the range of actions taken by the aquaculture industry to respond to concerns about welfare of fish on farms, it is hardly surprising that farmers are intensely irritated, and indeed often stamping in anger at the cynical repetition by a small number of professional campaigners, of half truths and outdated data. This young industry is progressively taking control of the process of growing fish and will continue to achieve improvement in fish welfare.

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Chapter 8
Stocking Density and the Welfare of Farmed Salmonids

James F. Turnbull, Ben P. North, Tim Ellis, Colin E. Adams, James Bron, Craig M. MacIntyre and Felicity A. Huntingford

Introduction

Our understanding of fish welfare is still very limited. Despite, or perhaps because of this lack of information, there is currently a great deal of interest in fish welfare. There is also an increasing pressure on legislators to protect the welfare of fish, the pressure for protection often outpacing scientific understanding of the nature of welfare, its assessment and control.

In terrestrial species, assessment of behaviour is an important source of information on welfare; for example the squeals made by piglets can be a reliable indication of how hungry they are (Weary & Fraser 1995). However, it is much more difficult to examine the behaviour of fish in production systems, therefore the evaluation and control of farmed fish welfare remains a challenge. What we do know is that fish welfare is affected by a range of complex interacting mechanisms. In the face of a poorly understood and difficult to observe system, one approach has been to look for simple production parameters, over which there is some capacity to exert control. Stocking density has been seized upon as one such parameter. The most basic formula for calculating stocking density is the total biomass of fish divided by the available space. However, this formula may not provide a true reflection of the experience of the fish in either cages or land-based systems since stocking density is only indirectly related to welfare. The relationship between stocking density and welfare is mediated through, or confounded by, a range of mechanisms including water quality, social interactions, food availability, contact with substrate and others. This paper presents a brief overview of the relationship between stocking density and farmed salmonid welfare.

Assessing fish welfare

The concept of welfare has many facets and the nature of welfare in relation to farmed fish is discussed elsewhere in this publication. Despite debate and

academic research, there is no universally accepted method of assessing farmed fish welfare. Simply measuring physiological responses is not enough. Even in humans it would be difficult to differentiate between pleasurable and fearful arousal by physiological analyses and even fearful arousal can be good or bad, for example riding on a rollercoaster compared with escaping from an attack. Dawkins (2004) proposed perhaps the most concise definition of welfare to date by suggesting that if the animals are healthy and have what they want then they have good welfare. Assessment of behaviour is an important source of information when deciding if animals have what they want (Weary & Fraser 1995) but unfortunately much of the behaviour of farmed fish is very difficult to observe due to the nature of the farming systems. Fish can often only be observed if they are in poor health or during feeding and that only provides us with limited information.

In both the commercial production and research environment, assessment of fish welfare is frequently limited to identifying fish with bad welfare, such as those with gross pathologies. There are also some indicators that are associated with an increased risk of deteriorating welfare, but our understanding of what constitutes good welfare for farmed fish is limited. Therefore, at present, welfare may be best measured using a range of indicators including health, growth and physiological parameters in an attempt to triangulate on the welfare status. This approach has its limitations since it is difficult to quantitatively relate such measurements to the welfare status of the fish and the relationship between indicators is not always constant.

Stocking density in cages

Commercial fish cage farms usually estimate the stocking density from a simple estimation of fish biomass and estimated net volume. However, the net cage is usually a flexible structure suspended in a rigid or semi-rigid collar. While the net is usually weighted to help maintain the structural integrity by minimising deformation, cages are subject to a wide range of deformation, resulting in a great deal of variability in the available space, especially in sites with high tidal energy or water velocity. Such deformation is also influenced by the accumulation of fouling organisms on the net (biofouling), which increases the weight and drag of the net. Biofouling also reduces water exchange and the fouling organisms themselves can have a direct effect on water quality, for example through consumption of oxygen or by filtering nutrients out of the water. Fouling organisms can also have a direct physical effect on the fish through abrasion, for example against molluscs.

Stocking density in tanks, ponds and raceways

The interaction between stocking density and welfare in rigid tanks, raceways and ponds is primarily influenced by a different set of variables. The volume available

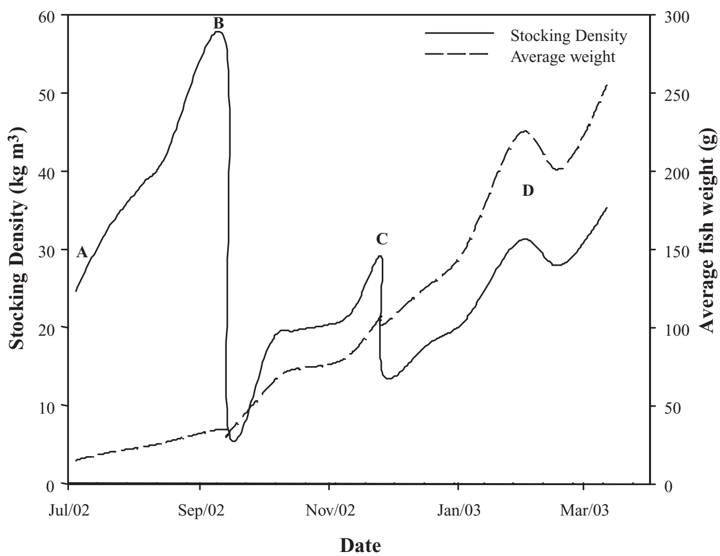

Figure 8.1 The variable stocking density experienced by rainbow trout during an on-growing cycle on a commercial farm (North 2004): (A) fish stocked in a raceway; (B) population split to reduce stocking density; (C) population transferred into larger on-growing unit; (D) selective harvest of largest fish resulting in a reduction in stocking density and average weight.

to the fish is obviously less variable. Biomass must still be based on estimates of weight and the number of individuals, but the rate of water exchange in such systems plays a far greater role. For this reason, a number of alternative methods for calculating the biological loading of the system have been proposed, however, in practice it is usually difficult to obtain accurate estimates of flow, and therefore biomass divided by volume is still the most commonly used and practical means of estimating stocking density (North *et al.* 2006). The alternative methods for calculating loading were summarised by Ellis *et al.* (2001).

Even when using a simple biomass/volume estimate of stocking density, the experience of the fish varies dramatically over the production cycle. Growth, grading and partial harvest can all have a dramatic effect on the concentration of fish within the system, as illustrated by the data from cultured rainbow trout, *Oncorhynchus mykiss*, presented in Figure 8.1.

Stocking density and behaviour

While our observations on freshwater behaviour are reasonably comprehensive, much of our understanding of the behaviour of salmon in the sea is based on opportunistic observations (Morgan *et al.* 1986, Dutil & Coutou 1988, Shearer 1992).

The spatial distribution that animals adopt in the rearing environment has been suggested as a valuable *in situ* indicator of how they respond to each other, and what they want in terms of space and stocking density (Dawkins 2004): an aggregated distribution indicates that the animals themselves choose to be in close proximity, a random distribution indicates that they show no preference, and a regularly spaced distribution indicates a degree of aversion to each other.

Rainbow trout have been observed to adopt an even distribution in tank systems (Buss *et al*. 1970, Heinen *et al*. 1996), which has been compared with repelling molecules of gases (Kils 1989). In contrast, rainbow trout have also been observed to aggregate within a sea cage (Phillips 1985).

Atlantic salmon (*Salmo salar*) in cages do not distribute themselves either randomly or evenly through the available space but school in dense groups (Juell & Fosseidengen 2004). The fish biomass in the volume actually occupied by fish has been referred to as the elective stocking density. A study using scanning hydroacoustics to quantify the location of the Atlantic salmon within production cages found that over a wide range of stocking densities (13–35 kg/m^3), the volume of cage used by the fish ranged from 45–85% of the available volume (Bell 2002). Neither the space occupied nor the inter-individual distance are affected by fish size (Juell & Fosseidengen 2004) but they are influenced by the number or biomass of fish, the available cage volume, temperature (Valdimarsson *et al*. 2000) and light (Juell & Fosseidengen 2004). The fish will also tend to segregate according to condition factor and swimming speed, which may be correlated (Boucher & Petrell 1997).

Regarding the schooling behaviour observed in cages, it would be useful to establish how groups of salmon behave in the wild at sea for comparison with their behaviour in cages. Although we know the destinations of wild Atlantic salmon marine migrations, their elective densities and the biological drives for schooling remain unknown.

Relationship between stocking density and welfare

An example of the challenges faced when examining the relationship between stocking density and welfare relates to the much-debated issue of farming Atlantic salmon in cages. It has been argued that confining a naturally migratory species may adversely affect their welfare; with proponents of this point of view citing the continuous circular swimming of salmon in cages as a form of stereotypy (Lymbery 2002). It has conversely been argued that fish in cages are protected from predators, supplied with easily available food and that their behaviour is simply an expression of their normal schooling behaviour. Compared with terrestrial animals, for example migratory mammals kept in zoos, we have very little information about the triggers for migration in fish. Do they migrate simply to find food or is there an alternative biological drive; and what are the welfare consequences of preventing migration? The answer is, we do not know.

The interaction between stocking density and fish welfare is very complex and variable even between individual tanks or cages, therefore it can be difficult to generalise from one situation to another. It is a task of considerable complexity to model the multiple interacting and confounding influences of stocking density on welfare and the many measurable aspects of welfare. The application of multivariate data reduction techniques and multivariable models has helped to tease information out of complex data sets. However, the statistical methods and computing power to deal with such complex data have only recently become widely available and therefore much of the literature relating to stocking density has dealt with simple univariate associations. Most of the relevant information also relates to the effects of stocking density in small-scale experiments with the focus on indicators of productivity without any explicit intention to investigate welfare. Many of the effects observed in these studies appeared to be study specific (Ellis *et al*. 2002). It is therefore difficult to extrapolate from this literature to the environment experienced in commercial farms.

The effects of stocking density on farmed salmonid fish appears to be mediated through water quality (reviewed by Ellis *et al*. 2002) and social interactions (MacLean *et al*. 2000, Adams *et al*. submitted). Not only high but also low stocking density can have a negative effect on fish welfare (Jørgensen *et al*. 1993, Adams *et al*. 1998). Farmers have recognised since the start of salmonid farming that damaging territorial behaviour in fresh water can be changed to schooling behaviour by increasing the stocking density. Once in a school, many aspects of behaviour are altered including reduction in aggression, but also risks taken during food acquisition (Grand & Dill 1999). Some of these changes may be beneficial for productivity and even improve welfare.

It has been suggested that increasing stocking density may increase the transmission or severity of infectious diseases within the tank or cage (Mazur *et al*. 1993, LaPatra *et al*. 1996, Bebak-Williams *et al*. 2002). Since fish at low densities still have ample opportunity to transmit infections by sharing the same water, and coming into contact during feeding and schooling, any association between increased fish density and infectious disease is likely to be highly complex. Controlled laboratory experiments have suggested that there are complex interactions between host density, pathogen density and individual group variation (Bebak-Williams *et al*. 2002). One trend that has been observed repeatedly is damage to fins as stocking density increases. This has been observed in both rainbow trout and Atlantic salmon (MacLean *et al*. 2000, Turnbull *et al*. 2005, North *et al*. 2006, see Chapter 9). There are several possible mechanisms by which fins could be damaged at higher stocking densities including increased aggressive interactions, contact with substrate or accidental damage during feeding.

One series of experiments demonstrated the complex determinants of welfare in farmed fish (Adams *et al*. 2007). Adult Atlantic salmon were held in seawater tanks at three stocking densities with five replicates per density and water quality was maintained at a uniformly high level. Four commonly-used measures of welfare

representing nutritional status, injury and stress level (body condition, fin condition, plasma concentration of glucose and cortisol) were combined by principal components analysis (PCA) into a single, integrated welfare score for each fish. Fish with high and low welfare scores were found in all density treatments, but welfare was best at the intermediate density, supporting the view that low densities as well as high densities can compromise welfare in Atlantic salmon. Disturbance (staff passing tanks) had a stronger effect on mean welfare score, and interacted with the effect of stocking density, creating a complex pattern of variation in welfare among the experimental tanks. Counter-intuitively, welfare scores were higher in tanks with a high level of disturbance. The frequency of aggression in all tanks was low, rising after feeding, when aggression was negatively related to both disturbance and stocking density. These data highlight the complex determinants of welfare and, taken together, suggest that when water quality is controlled at a high level, negative effects of husbandry practice and density on welfare in Atlantic salmon might be mediated by their influence on social interactions.

In a similar study of rainbow trout in freshwater tanks (North *et al*. 2006), stocking density did not significantly affect growth or mortality but increased stocking density did have a significant detrimental effect on fin condition. However, the fish held at lower densities had a significantly lower mean body condition factor and an increased size variation at the end of the study. Mean plasma cortisol levels were also higher at the lowest stocking density on five of the nine monthly sample points. The evidence for stronger dominance hierarchies in the low density treatment indicate that low, as well as high, stocking densities have the potential to adversely affect rainbow trout welfare. Furthermore, PCA also identified a group of fish common to all treatments that had very little dorsal and caudal fin damage, but low condition factors. This may have represented individuals that had adopted a non-competitive feeding strategy. This is evidence for alternative behavioural strategies within the population and raises the question of whether these fish with low body condition and better fins had better welfare than the rest of the population? The answer is probably that they had different, rather than better or worse welfare.

It can be argued that the only way to understand the welfare of farmed animals is to adopt an epidemiological approach and study them on commercial farms. A very large-scale study of chickens, conducted on 2.7 million birds, demonstrated that lowering stocking density in isolation did not have the expected benefits for welfare (Dawkins *et al.* 2004, Jones *et al*. 2005). The welfare experience of the chickens was related to a whole range of parameters, only one of which was stocking density. Real improvements in welfare required attention to a range of factors related to the farming system and the genetics of the chickens.

A study of Atlantic salmon in sea cages came to a similar conclusion (Turnbull *et al*. 2005). This study examined the welfare of over 248 000 Atlantic salmon in cages on a commercial marine farm, exposed to densities ranging from 9.7 to 34 kg/m^3. On three occasions over a period of 10 months, fish were sampled from

each cage, weighed and measured; their fin condition assessed and blood samples taken for measurement of glucose and cortisol. PCA was used to combine the four measures (condition of body and fins, plasma concentrations of glucose and cortisol) into a single welfare score. A statistical model indicated that the median welfare score for each cage was significantly related to sampling period, stocking density (averaged over the previous three months) and location of the cage relative to the shore. Further analysis of the relationship between stocking density and the welfare score suggested that there was no trend up to an inflection point around 22 kg/m^3, after which increasing stocking density was associated with lower welfare scores. This was a weak but statistically significant association. This study suggested that, while stocking density can affect the welfare of Atlantic salmon in production cages, it is just one of many factors that can influence their welfare and on its own cannot be used to accurately predict or to control welfare. Interestingly, the opinion of farmers at the start of this project was that stocking densities started to become problematic when they increased above the mid-20s kg/m^3. While not confirming the findings of the study, this does to some extent lend weight to the conclusions and also demonstrates that good farmers are aware of the welfare of their stock through close observation and practical experience, even if they do not necessarily think of this awareness in terms of fish welfare.

Should we control stocking density to protect fish welfare?

One of the challenges when discussing stocking density and welfare is effectively explaining the meaning of complex relationships to a non-scientific audience. It is often necessary to use analogies to explain the role of a factor that has a weak but significant effect on an outcome, such as stocking density and welfare. For example, if you already have a healthy diet and are not suffering from any form of debilitation or illness then vitamin supplements are unlikely to significantly improve your health. Neither will vitamin supplements protect you from the adverse effects of a diet low in fibre and high in fat and salt. However, vitamin supplements have a positive benefit in certain circumstances, for example during convalescence. Therefore the health of the population is more likely to be maintained through a good diet rather than through taking vitamin supplements. This is analogous to the association between stocking density and welfare. If the rest of the farming system is good then stocking densities can be relatively high with no adverse effect. If the rest of the system is bad then even acceptable lower stocking densities will not ensure the welfare of the fish. However, there are some circumstances such as high water temperature in cages where stocking density has to be controlled. The priority is to control those factors that are strongly associated with the outcome and, if resources are still available, then to deal with those factors that are associated with the outcome only weakly or under specific circumstances.

Conclusion

While it is still not exactly clear what constitutes good welfare for fish, the best way to avoid bad welfare would seem to be a combination of good general husbandry, health management, contingency planning to avoid system failures and humane slaughter. Good general management should include appropriate stocking density for the farming system but given the diversity of farms it is not feasible to prescribe exact densities that guarantee good welfare. Stocking density is an easy target for welfare pressure groups, but controlling stocking density is not the best way to protect fish welfare in all circumstances. It is also important to remember that farming practices are dictated not only by ethical concerns but also by economic realities. Therefore, changes of farming practices to protect fish welfare have to be economically viable, otherwise the welfare safeguards will not be implemented or the farming industry will cease to exist.

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Chapter 9

Fin Erosion in Farmed Fish

Tim Ellis, Birgit Oidtmann, Sophie St-Hilaire, James F. Turnbull, Ben P. North, Craig M. MacIntyre, John Nikolaidis, Imogen Hoyle, Steve C. Kestin and Toby G. Knowles

Introduction

Fin erosion refers to damage to and/or loss of fin tissue and is a well-known problem in many species of farmed fish. The presence of fin erosion is so ubiquitous in some species that it is used to determine whether the fish have farmed or wild origins (e.g. Butler *et al*. 2005). Due to its high prevalence, fin erosion has attracted a significant body of research as well as welfare attention. The purpose of this chapter is to review the current scientific information on fin erosion in farmed fish, relate it to fish welfare, and highlight knowledge gaps thereby building upon the recent review by Latremouille (2003).

What is fin erosion?

Fin erosion is a poorly defined condition. It refers to damage or degradation of the epidermis, dermis and fin rays of teleost fins resulting in a variety of effects including fraying, splitting, histological changes or a reduction in size (Sharples & Evans 1996, Latremouille 2003). Numerous other terms have been used to describe fin damage: e.g. fin rot, abrasion, lesions, wear and necrosis. The term fin erosion appears to have been used in the US to describe the condition in farmed trout since at least the early 1970s (Larmoyeux & Piper 1971), and has been widely used to date. However, the apparently analogous condition in farmed Atlantic salmon, *Salmo salar*, has traditionally been termed fin rot in the UK (e.g. Turnbull *et al*. 1996, 1998). The terms fin erosion and fin rot have similarly been used interchangeably to describe fin damage in wild fish (e.g. Minchew & Yarbrough 1977, Bucke *et al*. 1983, Lindesjöö & Thulin 1994, Wiklund & Bylund 1996).

Various authors have proposed clarifying the terminology and differentiating fin erosion from fin rot. Lindesjöö & Thulin (1990) suggested that the condition should be termed fin rot when there is a demonstrable infectious agent, and fin erosion if

no such agent is evident. Latremouille (2003) similarly suggested defining fin rot as the condition caused solely by bacteria, but expanded fin erosion to encompass any cause, including bacteria. The ability to apply appropriate terminologies is hampered by the facts that the aetiology of fin erosion is poorly understood and probably varied (see below), that the two proposed conditions are probably related, and that fin damage can also be a sign of systemic infection (Winton 2001).

Implicit in the term erosion, is that it is a gradual (chronic) process (Schneider & Nicholson 1980). Research on fin erosion experienced by farmed fish has shown that bacteria are not the primary cause, although secondary infections may be present (see below). The multifactorial aetiology (see below) indicates that fin erosion may cover a suite of different disorders. In fin erosion, the tissue degradation is restricted to the fins, the fish are typically otherwise in good condition (Plate 1) and it does not cause significant mortality. There is currently no evidence that fin erosion is transmissible.

Fin rot (sometimes referred to as tail rot) is a bacterial infection that causes fin damage and is known from a number of marine and freshwater species worldwide (Schäperclaus 1992). It is considered to be an acute, highly contagious condition, typically associated with high mortality. If the caudal fin degenerates the infection may pass to the caudal peduncle (Schäperclaus 1992). Fin rot has been attributed to a variety of bacteria (*Cytophaga, Flexibacter, Aeromonas, Pseudomonas, Vibrio*; Schäperclaus 1992, Bruno & Poppe 1996), and therefore again represents a suite of different disorders. These bacteria are typically facultative parasites, which only become pathogenic when fish health is compromised by unfavourable conditions. Fin rot has therefore been associated with physical damage, poor water quality and inappropriate nutrition (Schäperclaus 1992, Bruno & Poppe 1996).

Latremouille's (2003) definitions of fin erosion and fin rot therefore seem most suitable, as there may be a bacterial involvement in fin erosion, albeit as a localised secondary infection. However, it appears possible to differentiate fin erosion from fin rot, based upon transmissibility, acuteness and severity of effects on the fish. Another possible differential that merits further examination is that 'true fin rot' leads to a spiky, rather than smooth, appearance (Sedgwick 1985), due to persistence of the fin rays. Fin rot, however, probably represents an extreme on the continuum of fin erosion, as it is not attributable to a single obligate pathogen and the factors predisposing fish to fin rot are those that are thought to contribute to fin erosion (see below).

Which species are affected by fin erosion?

Fin erosion is best documented in farmed salmonids, and is known to occur in Atlantic salmon (*Salmo salar*), Pacific salmon (*Oncorhynchus* spp.), rainbow and steelhead trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), Arctic charr (*Salvelinus alpinus*), brook trout (*Salvelinus fontinalis*) and cutthroat trout (*Oncorhynchus clarki*) (e.g. Moring 1982, Jobling *et al*. 1993, Turnbull *et al*. 1996,

Bosakowski & Wagner 1994a, b). The marine flatfishes, Dover sole (*Solea solea*, Plate 2) and Senegalese sole (*Solea senegalensis*) are also prone to fin erosion, although accounts are limited (Smith 2003). Walleye (*Stizostedion vitreum*), a freshwater percid farmed for stocking fisheries in the US, have also been recorded as experiencing fin erosion (Clayton *et al.* 1998). However, there appear to be no documented accounts of fin erosion in other fish species farmed in Europe in fresh and seawater (e.g. turbot, sea bass, sparids, eel, carp, sturgeon, cod, halibut). Fin erosion is therefore not a universal problem for all farmed species, but it does occur in both marine and freshwater species and across different phylogenetic groups. There is no clear link between fin erosion and ecology: e.g. mid-water versus bottom living, or relative prey size. However, it has been implied that fin erosion may be a problem associated with farming of coldwater fish (Bosakowski & Wagner 1995).

How extensive a problem is fin erosion?

Although fin erosion appears to be a ubiquitous problem in certain susceptible commercially farmed species, there is a lack of quantitative information on its prevalence and severity. Qualitatively, fin erosion has been noted to be widespread within US trout farms (Bosakowski & Wagner 1994a, b) and is not uncommon on European trout farms (Sedgwick 1985). It has been recorded in commercial trout farms in Germany and the UK (Peters 1990, North 2004, St-Hilaire *et al.* 2006). It has been noted to occur in Atlantic salmon grown in Norway, Scotland and North America (Schneider & Nicholson 1980, Turnbull *et al.* 1996, Poppe 2000).

Fin erosion frequently affects most of the population of fish (Moring 1982). However, there is typically a large range in fin condition within a population (Lellis & Barrows 1997, St-Hilaire *et al.* 2006). It has been suggested that the smaller, less competitive individuals experience less erosion (Lellis & Barrows 1997). The dorsal fin can become 70% eroded in up to 95% of fish (Kindschi *et al.* 1991a). Complete loss of the dorsal and pectoral fins is also documented in a proportion of individuals (Heimer *et al.* 1985, Turnbull *et al.* 1996, St-Hilaire *et al.* 2006).

Given the lack of quantitative information it is impossible to determine whether the prevalence and severity of fin erosion is changing over time. However, Winfree *et al.* (1998) from the US noted that fin erosion is becoming more common in newer, more intensively managed farms, than in older and simpler culture systems such as earthen ponds. Poppe (2000) implied that fin erosion in European Atlantic salmon farming has increased with increased intensification.

How long has fin erosion been recognised as a problem?

Fin erosion has been recognised since the 1970s in rainbow trout (Larmoyeux & Piper 1971, Boydstun & Hopelain 1977) and Atlantic salmon (although termed fin

rot – Schneider & Nicholson 1980). There has been significant research into fin erosion in the US because it affects the aesthetic quality and possibly the ability of stocked fish to survive in the wild (Bosakowski & Wagner 1994b). Fin erosion has not attracted as much specific research attention in Europe (apart from Turnbull *et al*. 1996, 1998) This is presumably because it does not appear to be a transmissible disease, and although it can affect the aesthetic quality of table fish (Rønsholdt & Mclean 1999), it is not an important determinant of carcass quality (it is not referred to in Kestin & Warriss 2001).

It appears that fin erosion was first highlighted as a fish welfare issue in Germany in 1990 (Peters 1990), but was not picked up in the early discussions on fish welfare in the UK (Lymbery 1992, Kestin 1994). However, in 1996 the UK's Farm Animal Welfare Council's report (FAWC 1996) noted fin damage in both Atlantic salmon and rainbow trout and suggested that it was linked to poor handling and inappropriate stocking densities. Subsequently, Turnbull *et al*. (1998) suggested that it was a welfare concern within the salmon industry, and it has been mentioned in popular press articles (e.g. Sandison 2000).

In 2000, fin erosion was implied to be a husbandry disease/production disorder related to intensification of the fish farming industry and thus affected the public perception of the health of farmed fish (Lellis & Barrows 2000, Poppe 2000). In the In Too Deep report (Lymbery 2002), fin damage was noted from salmon and trout and explicitly linked to welfare, and was suggested to be caused by abrasion with the sides of cages or conspecifics. Fin erosion is increasingly being linked to fish welfare within the scientific community (e.g. Ellis *et al*. 2002, Turnbull *et al*. 2005, Huntingford *et al*. 2006). Nevertheless, an objective discussion of fin erosion as a welfare issue is currently lacking.

How is fin erosion assessed?

Latremouille (2003) reviewed methods for the assessment of fin erosion, which fall into seven categories. The first four describe the state of the fin, while 5–7 attempt to quantify the amount of tissue lost.

(1) *Macroscopic descriptions of fin state.* Goede & Barton (1990) proposed a descriptive scale that has been adapted by Adams *et al*. (1993) and Tierney & Farell (2004). These three- or four-point scales classify fins as intact, healed, undergoing minor active erosion or severe active erosion with haemorrhaging. Turnbull *et al*. (1996) classified dorsal fin erosion into seven categories based upon appearance and tissue loss: peripheral erosion and ray splitting; peripheral erosion with some nodularity; severe nodularity with tissue loss; extensive fin loss; smooth thickening; haemorrhagic lesions; and healed lesions.

(2) *Description of fraying/splitting*. Bosakowski & Wagner (1995) recorded whether fins were split or frayed and expressed this as a frequency. MacLean

et al. (2000) used a three-point scale to describe splitting as either absent, mild or severe.

(3) *Thickening*. MacLean *et al.* (2000) used a three-point scale to describe thickening as either absent, mild or severe.

(4) *Microscopic descriptions of fin state*. Bodammer (2000) classified fin erosion in wild fish into four categories: epithelial cell hyperplasia; mucous cell hyperplasia and hypertrophy; spongiosis; and focal necrosis.

(5) *Descriptions of the amount of fin tissue lost*. Scales for visual assessment of the area of fin lost have been widely used due to their simplicity: e.g. Bosakowski & Wagner (1994a) used a three-point scale to classify fins as perfect, slightly eroded or severely eroded; Moutou *et al.* (1998) used a four-point scale – no damage, minor (<30% fin tissue lost), severe (30–70% tissue loss) and very severe (>70% loss) damage. MacLean *et al.* (2000) used a five-point scale.

(6) *Measurements of fin length*. Kindschi (1987) proposed using fin length expressed as a percentage of fish total length, i.e. the fin index, to quantify the extent of fin erosion. Bosakowski & Wagner (1994b) further refined Kindschi's fin index by defining fin length as maximum fin length parallel to the fin rays, and statistically demonstrating that the relative size of fins did not change with fish size.

(7) *Measurements of fin area*. With the development of image analysis techniques and digital photography, accurate quantification of fin area is possible (Smith 2003, North 2004). However, this method requires standardised splaying of fins, and has only been used for median, rather than paired fins, as contrast with the background is needed.

As discussed by Latremouille (2003), the various assessment methods have different merits in terms of assessment time, sensitivity, and ability to discriminate ongoing from healed damage. It must be remembered that the appearance and size of the fins will be the result of recent damage, historical damage, the healing response of the tissue, and regrowth (Turnbull *et al.* 1996).

A significant problem in using descriptive and scale methods is the inherent subjectivity. For example, Wagner *et al.* (1996a, b, c) used Goede & Barton's (1990) scale, but judged severity on fin size in addition to visual appearance. Fin length measurements largely remove this subjectivity although the actual measurements of fin length do need to be standardised. Latremouille's (2003) definition of fin length – the median point of attachment to a fish's body to the most distal median point of the fin – differs to that of Bosakowski & Wagner (1994b). Clayton *et al.* (1998) determined caudal fin length from the difference between total and standard length. Clayton *et al.* (1998), Pelis & McCormick (2003) and St-Hilaire *et al.* (2006) expressed fin length as a percentage of standard or fork (rather than total) length to overcome the problem of erosion of the caudal fin.

As well as the variety of methods that have been used to assess erosion, the analyses differ considerably between studies. Many studies have assessed erosion

in selected fins (e.g. just the dorsal), whilst others have looked at all fins. In the latter case, some studies have assessed values for the individual fins separately (e.g. St-Hilaire *et al*. 2006), whilst others have pooled values of all fins on a fish to examine factors that contribute to overall fin erosion (e.g. Bosakowski & Wagner 1994a). These differences in assessment and analysis methods make comparisons between studies extremely difficult.

Information is available on the lengths of fins of wild rainbow trout (Kindschi *et al*. 1991b, Bosakowski & Wagner 1994b, Pelis & McCormick 2003) which theoretically provide a true 'intact' value. However, caution does need to be applied in interpretation. The mean dorsal fin index of wild rainbow trout (13% of fish length) recorded by Bosakowski & Wagner (1994b) is greater than that of intact dorsal fins of wild and reared fish (10–11%) recorded by Kindschi *et al*. (1991b). Developmental plasticity and genetic differences may lead to differences in the size of intact fins (e.g. Dynes *et al*. 1999, Imre *et al*. 2002, Pelis & McCormick 2003), and the assumption of isometric growth of fins and the body needs further validation. The size range of rainbow trout assessed by Bosakowski & Wagner (1994b) was limited (113–285 mm), and the validity of their statistical analysis demonstrating isometric growth over this range is uncertain. In their regression analysis of relative fin length (fin length/body length) against body length, they related two variables that were not independent, and they used least squares rather than functional regression which may be more appropriate (Ricker 1973). Pelis & McCormick (2003) recently assessed fin size in a larger number of Atlantic salmon parr ($n = 120$) and smolt ($n = 24$) of a wider size range. They found that fin growth was isometric in the median fins, but the relative size of the paired fins did change with fish size (i.e. allometric growth).

Which fins are affected?

Teleosts typically have seven rayed fins: the median dorsal (D), caudal (C) and anal (A) fins and the paired pectoral (P_1) and pelvic (P_2 = ventral) fins (Plate 1). Salmonid fish also have an additional non-rayed adipose fin. It is well documented that the different fins are differentially prone to erosion. These differences may reflect differences between the individual fins in the level of damage received, their robustness, or their response to damage (Turnbull *et al*. 1998).

The adipose fin does not appear to erode in salmonids, whereas all the rayed fins experience erosion to various degrees (Bosakowski & Wagner 1994b). There is a general pattern in salmonids with the D and P_1 fins subject to the most damage, although the exact order differs between studies. Turnbull *et al.* (1998) observed $D > P_1 > C > P_2 > A$ in Atlantic salmon parr. Pelis & McCormick (2003) observed $D = P_1 > A > P_2 > C$ and $P_1 > D > P_2 > A > C$ in two different Atlantic salmon parr hatcheries. St-Hilaire *et al*. (2006) observed $D > P_1 > C > A > P_2$ in rainbow trout sampled from UK rainbow trout farms. Bosakowski & Wagner (1994b) observed

D > P_1 > A > P_2 > C in rainbow and brown trout, and P_1 > D > A > P_2 > C in cutthroat trout. Abbott & Dill (1985) observed D > P_1 > C > P_2 > A in juvenile steelhead. The first study assessed tissue damage from the length of the splits in the fins, the second, third and fourth studies assessed tissue loss by comparison of fin lengths with those of control (feral or wild) fish, and the last study assessed tissue loss by subjective classification of damage.

Fin erosion in Dover sole differs to that in salmonids, in that it is the C fin that is most affected by erosion, with the marginal (D and A) fins affected somewhat less (Smith 2003). In walleye, the P_1 and P_2 fins were the most eroded, followed by the C fin (Clayton *et al*. 1998).

When does fin erosion develop?

There has been little quantitative work on the ontogeny of fin erosion in farmed fish. St-Hilaire *et al.* (2006) compared the relative fin sizes of rainbow trout that had recently arrived (<30 g) on farms growing fish for the table (rather than the stocking/angling market), with fish that had been held on the same farms for a significant period (>100 g), and used feral fish as a control. They concluded that the bulk of the damage to the D and P_1 fins had occurred earlier in the production cycle in the hatchery, although tissue loss from these fins did continue on the table farm. In contrast, the bulk of the erosion of the C, A and P_2 fins was considered to occur on the table farm. There therefore appears to be a difference in the timing of erosion of the different fins.

Two studies have made a more detailed qualitative assessment of the progression of D fin erosion. Kindschi *et al*. (1991b) visually examined rainbow trout fry, and found that damage started at around 35 mm in length with tears between the fin rays of up to half the length of the fin. This was followed by tissue loss from the fin margin which progressed as the fish grew. Turnbull *et al*. (1996) compared the state of D fins in batches of Atlantic salmon parr and similarly observed that fin damage was initiated with splitting between the fin rays and clefts through the epithelium that was followed by increasing degrees of tissue loss from the fin margin. It is thought that once damage is initiated, it continues through the culture cycle (Barrows & Lellis 1999).

Is fin erosion a welfare issue?

The various morphological changes evident in damaged fins are not thought to affect the development or feeding ability of the fish (Bruno 1990). However, due to a lack of directed research and the diversity of the condition, it is currently difficult to determine whether fin erosion does represent a benign condition, or whether it is a significant welfare issue. Nevertheless, the potential impacts of fin erosion can be

discussed by using one of the five freedoms (see Chapter 2) as a basis for welfare assessment of fish (Huntingford *et al*. 2006): i.e. freedom from injury, disease and functional impairment.

Injury

The typical structure of a rayed teleost fin is a layer of skin (epidermal cells overlying the dermis) supported by skeletal elements (the fin rays) that are connected to each other and surrounding tissues by collagenous ligaments (Becerra *et al*. 1983). The fin rays are calcified tubes composed of two hemispherical tubes that contain blood vessels and nerve bundles (Becerra *et al*. 1983, Plate 3). Fins are therefore composed of live tissue, and are not analogous to mammalian hair or nails. Fin erosion can therefore be classed as injury, as it represents damage to live tissue.

The term fin erosion encompasses a variety of types of tissue damage: e.g. splitting, epithelial clefts, necrotic tissue, and sloughing (Turnbull *et al*. 1996). As blood vessels are present, fin damage can result in haemorrhaging. Damaged fin tissue will show an inflammatory response that can result in epithelial hyperplasia, thickening and nodularity (Turnbull *et al*. 1996).

As fin damage represents injury to live tissue, the potential exists for pain. As highlighted by Sneddon in Chapter 4, it is now well documented that fish are capable of nociception, although debate continues as to whether fish have the capacity to undergo the psychological experience of suffering pain as in humans (Rose 2002, Chandroo *et al*. 2004). Although the recent work on nociception in fish has focused on the head of the rainbow trout, some relevant information does exist for fins. Nerve bundles are known to be present within the fin rays from histological studies (Becerra *et al*. 1983, Plate 3). The presence of a nervous supply to fins has additionally been demonstrated by fin regeneration studies, as regeneration requires a nervous supply (Weis 1972, Geraudie & Singer 1985). Chervova (1997) demonstrated experimentally that fins are capable of nociception. He applied noxious stimuli (skin punctures, compression, electrical stimulation) to the fins and other areas of the skin of steelhead trout and cod, and interpreted a tail jerk as a nociceptive response. He interpreted his results as illustrating that the fins (C, D, P_1) were the most 'sensitive' regions of body. Additionally, he demonstrated that analgesics (dermorphin and β-casomorphine) increased the response threshold of the fins to the noxious stimuli.

Disease

The role of pathogens in fin erosion (as opposed to fin rot) is unclear (discussed below). The breakdown in the epithelial barrier during active fin erosion may facilitate secondary infection by facultative bacteria, which have been recorded in eroded fins (see below). The barrier breakdown may also facilitate the entry of other systemic infections. For example, the fins are a known route of entry for

infectious haematopoietic necrosis virus (IHNV), *Flavobacterium psychrophilum* (the causative agent of rainbow trout fry syndrome (RTFS) and bacterial coldwater disease) and the parasite *Myxobolus cerebralis* (the causative agent of salmonid whirling disease) (El-Matbouli *et al*. 1995, Martinez *et al*. 2004, Harmache *et al*. 2006). However, it remains to be demonstrated whether fin erosion does increase susceptibility to such infections.

In the absence of a clear infective agent, whether fin erosion can be classed as a disease in its own right depends upon how disease is defined. Active fin erosion represents a pathological change in the structure of fins, characterised by clearly identifiable signs, and would therefore generally be classed as a disease. Healed fins that are abnormal in structure and size with a potentially affected function (see below), may also be classed as a disease using broader definitions.

Functional impairment – physiological effects

Very little information is available on the physiological effects of fin erosion on fish. It has been suggested that fin damage may cause stress and osmotic disturbance and incur an energetic cost due to repair, thereby decreasing 'vigour' and growth (Abbott & Dill 1985, Turnbull *et al*. 1996, Clayton *et al*. 1998). However, there is currently no evidence for these hypotheses. A single study has indicated that various biochemical processes in the red blood cells are altered in Atlantic salmon experiencing fin damage (Tret'yakov *et al*. 1988), although it is unclear whether these fish were experiencing fin erosion or bacterial fin rot.

Functional impairment – behavioural effects

The primary function of the fins is for locomotion. Each fin ray is moved by an individual set of muscles, allowing fins to be spread, collapsed or undulated, with the paired fins additionally capable of forward and backward movement (Marshall 1965). The fins are therefore capable of a range of movements and are used for stabilising, manoeuvring and braking, as well as providing propulsion, with the different fins performing different roles (Drucker & Lauder 2003, 2005, Pelis & McCormick 2003).

The median fins can also play an important function in preventing displacement by water currents when the fish are resting on the substratum. Stationary Atlantic salmon parr lying on the substratum extend the pectoral and pelvic fins which, due to the hydrofoil action, provide a downward force (Arnold *et al*. 1991). Atlantic salmon parr have also been observed to grasp gravel between the pelvic fins to aid station-holding (Arnold *et al*. 1991).

A secondary function of fins in some species is intraspecific communication. Salmonids are believed to use erection and depression of the dorsal fin to visually communicate aggressive and submissive intent to conspecifics (see Abbott & Dill 1985). The loss of fin tissue may theoretically impede such behaviour. The intact

dorsal fin of rainbow trout has a white distal margin that is believed to be part of the visual signal and may stimulate aggressive behaviour (Abbott & Dill 1985). It has been suggested that the abnormal white epithelium associated with eroded dorsal fins may prompt attack due to misinterpretation as an aggressive/dominant signal (Wagner *et al*. 1996c), thereby leading to increased aggression. The distinct abnormal white tissue has also been suggested to increase visibility to predators (Abbott & Dill 1985).

A third potential function of fins is gaining sensory information about the environment. Taste buds have been observed on the fins of cod (Harvey & Batty 1998), although how prevalent these and other sensory organs are on other species is, as yet, undocumented. Chervova (1997) described the fins as one of the most sensitive nociceptive area of fishes, so it is possible that they do perform additional sensory (e.g. tactile) roles.

It has been suggested that fin erosion may reduce the range of fin movement, decrease manoeuvrability and swimming performance, cause fish to adopt body positions to protect injured fins and lead to increased susceptibility to predation, reducing feeding efficiency and displacement into low flow areas (Abbott & Dill 1985, Turnbull *et al*. 1996, Barthel *et al.* 2003, Huntingford *et al*. 2006). However, such proposed effects are speculative (Barrows & Lellis 1999), and the limited evidence currently available indicates that eroded fins do not affect behavioural performance. Berejikian & Tezak (2005) found that dorsal fin size did not impact on the ability to achieve dominance in aggressive interactions. A single study examining the effect of eroded pectoral fins found no effects on post-release survival, growth or distribution (Heimer *et al*. 1985).

Nevertheless, circumstantial support for a possible effect of fin erosion on behavioural performance comes from studies on fin-clipped fish, some of which have shown an effect on survival in the wild (e.g. Coble 1971, McNeil & Crossman 1979, Johnsen & Ugedal 1988, Pratt & Fox 2002). Amputation experiments have shown that loss of the pectoral fin does reduce the ability of Atlantic salmon to rest on the bottom when exposed to currents (Arnold *et al*. 1991), and that loss of the adipose fin causes an increase in caudal fin movements (Reimchen & Temple 2004).

What causes fin erosion?

There has been a significant research effort in the US directed at identifying the aetiology of fin erosion due to its perceived importance to the aesthetic appeal and performance of stocked fish. However, fin erosion has proved to be a complex multifactorial process and remains poorly understood. Evidence exists to show that a variety of primary and secondary causes contribute to erosion. Secondary factors may not act directly to cause damage, but indirectly by impeding healing and regeneration. Determining the aetiology is further complicated by differences between fins in the extent and timing of damage. These differences are presumably

due to the siting of the fins on the fish, and indicate that localised, physical factors are the primary causes of fin damage. More generalised environmental factors therefore presumably act as secondary factors.

Nipping – aggressive and accidental

There is good experimental evidence that aggressive nipping is a primary cause of fin erosion. Abbott & Dill (1985) observed aggressive nips – associated with fights and establishment of dominance hierarchies – in juvenile steelhead trout. These nips were aimed at all areas of the body, but nips were generally orientated downwards and hence directed at the D, P_1 and C fins rather than the A and P_2 fins. Evidence that nips did result in fin damage came from the relationship between the relative frequencies of aggressive nipping and observed damage. Turnbull *et al.* (1998) made similar observations in Atlantic salmon parr, showing that all body areas were subject to aggressive nipping, but that the attacks again tended to be directed at the D and C fins. Turnbull *et al.* (1996) further observed clefts in the epithelium of the D fin that were considered to correspond to tooth puncture marks.

It is possible that accidental nipping occurs during feeding with conspecifics being bitten in mistake for food pellets. Although this possibility has not been examined, it has been suggested that fish that compete less for food experience less erosion (Lellis & Barrows 1997). The observation that larger fish suffer more dorsal fin damage has been confirmed in Atlantic salmon, although the authors suggested it was due to aggressive nipping between the dominant individuals who were aggressively competing for food (MacLean *et al.* 2000).

Handling and transport

Handling has been proposed as a cause of fin erosion (FAWC 1996). Handling damage encompasses tissue trauma that may be caused by physical contact with surfaces (e.g. nets, fish pumps, grading machines, vaccination tables), by contact with other fish within nets and during crowding within tanks (e.g. prior to processing or when tanks are drained for cleaning) (Kindschi *et al.* 1991a) or during manual handling (e.g. vaccination, hand-grading). Transport will involve 'handling' fish at both loading and unloading, and fish bumping into the walls of the transport tank may additionally induce damage. Unfortunately, very little information is available to make an accurate assessment of the role of handling in fin erosion.

In an angling study with bluegill (*Lepomis macrochirus*), Barthel *et al.* (2003) found that containment of individual fish within a landing net for 30 seconds caused splits in the P_1 and C fins (the only ones examined). There was evidence that the mesh type (e.g. knotless versus knotted) and mesh size affected the frequency of damage, and it was suggested that the level of 'thrashing' within the net and size of the fish would further affect such damage. Whether containment of larger numbers of fish within a net, as occurs during farming, would increase such damage, is

unknown. Helfrich *et al*. (2004) found little evidence that passage of fish through a fish pump caused fin damage. Schäperclaus (1992) reported that transport caused physical damage to fins, although it is unclear whether such damage occurred during transportation, or during the capture, loading or unloading. Iversen *et al*. (2005) recently demonstrated that roughness of transport affected the stress level of Atlantic salmon, but fin damage was not examined.

Abrasion – tank surfaces and fish

Abrasion against the sides of the rearing unit was originally thought to be the cause of fin damage in farmed fish, hence the adoption of the term erosion (Abbott & Dill 1985). However, Abbott & Dill (1985) questioned this interpretation, as abrasion is unlikely to cause erosion of the D fin due to its position on the fish. There is currently no direct observational evidence that abrasion is a cause of fin damage, although indirect evidence is available.

Turnbull *et al*. (1998) suggested abrasion to explain the observation of P_1 fin erosion in salmon held in isolation, and that of communally held fish experiencing more erosion than expected from the relative frequency of aggressive nipping attacks. Bosakowski & Wagner (1994a) found that fin size of fish in commercial US hatcheries was affected by the unit construction – fish reared in concrete and steel raceways had smaller fins than those reared in systems with more natural substrata (i.e. gravel/earth ponds). This was interpreted as an effect of the substratum, although system type may have been a confounding factor. Bosakowski & Wagner (1994c) went on to demonstrate experimentally that addition of a cobble (2–4 cm diameter) substratum in raceways reduced erosion of the C, A, P_1 and P_2 fins of trout, which they concluded demonstrated the involvement of abrasion in the erosion process. Wagner *et al*. (1996c) similarly demonstrated reduced erosion of the C, A, P_1 and P_2 fins as well as the D fin when the floor of the raceway was covered in cobble and gravel, although they did not elaborate on the role of abrasion. Barnes *et al*. (1996) found that insertion of baffles into raceways increased erosion of the P_1 and P_2 fins, which was interpreted as being due to increased abrasion with the raceway floor. Arndt *et al*. (2001) again found that provision of a gravel substratum reduced erosion of all fins. However, this study cast doubt on the role of abrasion, as a resin coating on concrete raceways did not reduce erosion. It is possible that associated changes in water flow dynamics, fish distribution, orientation, or swimming and feeding behaviour are the proximal cause of treatment effects in the substratum/baffle experiments.

Asymmetric erosion of paired (P_1 and P_2) fins has been observed in trout held in smooth walled fibreglass circular tanks (pers. obs.), which had initially been attributed to abrasion damage. In such systems, the fish typically swim in a circular pattern, orientating into a current induced by the water inflow to aid with tank self-cleaning. However, experimental observations have demonstrated that it is not the outside fin near the tank wall which is subject to the greatest degree of erosion, but

the inside fin. This indicates that abrasion with the tank side is not the reason for the asymmetric erosion observed in such systems.

Further suggestions are that abrasion damage may occur in episodic (rather than continuous) collisions with the rearing unit when fish are startled (Boydstun & Hopelain 1977) via fish–fish contact when fish are aggregated during feeding or around water inlets (Winfree *et al*. 1998) or might impede healing rather than causing damage (Bosakowski & Wagner, 1995). Again there is currently no evidence for these alternative suggestions.

Sunburn

Sunburn is frequently suggested as a potential cause of fin erosion for those fins that may be exposed to ultraviolet light. Kindschi *et al*. (1991a) noted an apparent correlation to sunlight intensity as D fin erosion developed after fish were moved outside in July/August and decreased in late September. There is a significant body of evidence that fish do experience sunburn, i.e. epidermal degeneration caused by ultraviolet light (Bullock 1988). Degeneration of the D, P_1 and upper lobe of the C fin has been observed in commercially farmed Atlantic salmon and rainbow trout fry and attributed to solar radiation from histological examination and reduction of the erosion following shading (Bullock 1988).

However, doubt has been cast on the role of sunburn. Wagner & Bosakowski (1994) found no effect of shading on fin erosion in rainbow trout. Although Wagner *et al*. (1995) found that covers did reduce erosion of the P_1 fins in cutthroat trout, this was interpreted to be due to behavioural changes associated with altered light levels. Kindschi *et al*. (1991b) found that trout held individually outside and exposed to sunlight did not experience D fin erosion, whereas unshaded production fish did.

Water quality

Fin erosion has been anecdotally related to low levels of dissolved oxygen and high levels of suspended solids (Wedemeyer 1996), although experimental and/or field evidence is lacking. Gas supersaturation is known to cause gas bubble disease, which often affects the fins (Wedemeyer 1996), and it has been suggested that this may initiate fin erosion.

In their field survey of US trout farms, Bosakowski & Wagner (1994a) found that lower alkalinity and higher ammonia levels were correlated to increased severity of erosion. The mode of action of alkalinity and ammonia in contributing to fin damage were, however, not established. North (2004) found a similar correlation between fin erosion and un-ionised ammonia concentration in UK trout farms, although no link was apparent with alkalinity. Nevertheless, water quality is unlikely to be a primary cause of fin erosion, as Kindschi *et al*. (1991b) found that isolated fish held in the outflow water from production tanks containing fish with eroded fins did not suffer from erosion.

Infection

Bacteria have been observed microscopically and have been isolated by culture from samples of eroded fins (Schneider & Nicholson 1980, Turnbull *et al.* 1996). Schneider & Nicholson (1980) found that bacteria were present in much greater numbers on eroding fins than on healthy fins of Atlantic salmon. They recorded a variety of bacteria with *Flexibacter* sp. and *Aeromonas salmonicida* being predominant. Turnbull *et al.* (1996) also isolated *A. salmonicida* from some eroding salmon parr fins, which were associated with haemorrhagic lesions and furunculosis. Bacteria, therefore, do play a role in fin erosion. However, two separate studies on Atlantic salmon and rainbow trout (Turnbull *et al.* 1996, Winfree *et al.* 1998) examining the prevalence and distribution of bacteria have concluded that bacterial infection is a secondary, rather than the primary, cause of fin erosion. The finding of Kindschi *et al.* (1991b) that fish did not suffer from erosion when held in the outflow water from production tanks containing fish with eroded fins, further indicates that a transmissible infective agent is not a primary cause. Chemotherapeutants have been found to improve fin condition, albeit to a limited extent in some, but not all, studies (see Sedgwick 1985, Kindschi *et al.* 1991b, Winfree *et al.* 1998, Latremouille 2003) again indicating that infections are a secondary cause.

Infection by opportunistic fungi has been suggested (Bosakowski & Wagner 1994a) but has yet to be demonstrated. Fins are known as a site of attachment for a variety of external parasites, and fin erosion has been related to ciliate, sea lice and *Gyrodactylus* spp. infections (Schäperclaus 1992, Rahkonen 1994, Dawson 1998). However, such ectoparasites have not generally been associated with eroded fins in experimental studies.

Nutritional quality of the diet

There is a substantial literature demonstrating that diet quality can affect fin erosion (see Latremouille 2003). During early development of commercial fish feeds it was recognised that the levels of various dietary components, e.g. fatty acids (e.g. Castell *et al.* 1972), amino acids (e.g. Ketola 1983), vitamins (e.g. Woodward 1984) and minerals (Ogino & Yang 1980) affected fin erosion. However, there does appear to be room for improvement to current fishmeal-based diets. Recent studies have shown that dorsal fin erosion is almost eliminated when trout are fed a krillmeal, rather than a fishmeal-based diet (Lellis & Barrows 1997, Barrows & Lellis 1999). Further studies by the same group have shown that trout fed commercial diets experience greater dorsal fin erosion than fish fed novel formulations (Lellis & Barrows 2000). These effects were thought to be mediated through a direct nutritional effect, e.g. by the protein composition or mineral balance affecting initial fin development or regeneration (Barrows & Lellis 1999). Nevertheless, nutrition is unlikely to be a primary cause of erosion – fish held in isolation but fed the same

diet as communally held fish did not experience the erosion of the latter group (Kindschi *et al*. 1991b, Turnbull *et al*. 1998). Kindschi *et al*. (1991b) raised the question whether aggressive nipping was due to nutritional deficiencies; if true, then dietary improvements would reduce nipping.

Stress

Kindschi *et al*. (1991b) indicated stress as a potential cause of fin erosion. Udomkusonsri *et al*. (2004) have recently suggested that stress, induced by confinement, caused an ulceration response in the fins of hybrid striped bass (*Morone saxatilis* x *M. chrysops*) affecting the epidermal and dermal layers within 15 minutes. They observed differences in ulceration between fins, with the C, D and P_1 fins affected most, followed by the P_2 and A fins. They suggested, based on observations of behaviour, that these lesions were not induced by physical trauma during the confinement but were induced by the stress itself. They suggested that this stress response may be a causal factor in fin erosion and went on to show that such ulceration could enable *Saprolegnia* infection (Udomkusonsri & Noga 2005). However, they could not reproduce this ulceration response in rainbow trout, although they suggested it might have occurred if environmental conditions (e.g. temperature) were different (Udomkusonsri & Noga 2005). Although stress remains to be demonstrated as a primary cause, it is a probable secondary factor, as stress hormones have been shown to affect the skin structure and rate of dermal repair in salmonids (Roubal & Bullock 1988, Iger *et al*. 1995). Chronic elevation of cortisol levels has also been linked to increased susceptibility to bacterial and fungal infections including fin rot (Pickering & Pottinger 1985).

What about healing and regeneration?

Damaged fins are capable of healing. Healing of the damaged epithelium initially occurs within 1–24 hours by migration of epithelial cells to form a 2–3 cell layer of nearly normal thickness covering the wound (see Goss & Stagg 1957, Kurokawa *et al*. 1993, Turnbull *et al*. 1996). Epidermal thickening has been observed in surgically amputated fins prior to fins regaining a more normal form (Goss & Stagg 1957), and is documented in farmed fish experiencing fin erosion (Turnbull *et al*. 1996). Repair to the underlying tissues then occurs through chronic inflammation and a severe fibro-cellular reaction (Turnbull *et al*. 1996).

Fins are also capable of regenerating lost tissue. Regeneration has been demonstrated by studies of fin clipping/amputation, although it is unclear whether fins do regrow to the original size, form and colouration (Tassava & Goss 1966, Weis 1972, McNeil & Crossman 1979). Regeneration after partial amputation takes a few weeks to months to complete, although the time required depends upon the amount of tissue initially removed and the size/age of the fish (Tassava & Goss 1966, Weis

1972, Santamaría *et al*. 1992). Regeneration occurs from the distal edge of the fin through the addition of new ray segments (Goss & Stagg 1957, Santamaría *et al*. 1992). Apical blastema form, from which cells differentiate to form the fin rays (lepidotrichia) and the surrounding tissue, which are reinforced by collagenous ligaments (Santamaría *et al*. 1992). Fins do not regenerate if completely removed, as regeneration requires cells derived from pre-existing rays (Goss & Stagg 1957, Bosakowski & Wagner 1994b).

It is not known whether the gradual erosion of the fins of farmed fish (as opposed to a neat clip) affects regrowth, as there have been no studies examining the regeneration of eroded fins. The rates of wound healing and fin regeneration are known to be affected by water chemistry, with research focused on toxicants such as heavy metals and pesticides (e.g. Verma 2005).

What else affects fin erosion?

The severity of fin erosion is thought to be influenced by a variety of biotic and abiotic environmental risk factors. These are presumed to affect the rates of erosion and healing/regeneration. It is important to gain an understanding of such risk factors for fin erosion because, if these are within the farmers' control, fin erosion can potentially be reduced.

Stocking density

Animal welfare pressure groups have linked fin erosion to stocking density (FAWC 1996, Lymbery 2002). There is also a significant body of scientific evidence from both the field and laboratory experiments demonstrating that increasing density (i.e. an increase in number or biomass per unit volume) can increase fin erosion (see Bosakowski & Wagner 1994a and Ellis *et al*. 2002 for discussions of Atlantic salmon and rainbow trout; Wagner *et al*. 1997 for cutthroat trout). Fin erosion in Atlantic salmon has been related to increased intensification (Poppe 2000), and a link between density and fin erosion has recently been noted on rainbow trout farms in the UK (North 2004).

Nevertheless, the linkage between density and fin erosion may not be as strong as has been intimated by pressure groups. Bosakowski & Wagner's (1994a) analysis of US trout farms indicated that three other factors (water alkalinity, unit construction and ammonia) were more significant factors for fin erosion than stocking density. Not all experimental studies have found an adverse effect of density (see Bosakowski & Wagner 1994a, Ellis *et al*. 2002, Latremouille 2003). Fish have been sampled from commercial UK trout farms operating at high densities with very good fins (North 2004). That higher density does not intrinsically increase fin erosion is shown by studies on Arctic charr, in which fin damage decreases with increasing density (Siikavuopio & Jobling 1995).

It cannot, therefore, be assumed that higher densities increase erosion. How density acts to increase fin erosion is unknown – suggestions include behavioural changes, water quality deterioration, and increased bacterial loading (Bosakowski & Wagner 1994a). Other factors influencing fish behaviour or water quality may therefore override an effect of stocking density, as exemplified by Arctic charr.

Unit construction and design

There is much evidence that fin erosion is influenced by various adaptations to system design, substratum, and structural complexity (Bosakowski & Wagner 1995, Barnes *et al*. 1996, Wagner *et al*. 1996c, Arndt *et al*. 2001, 2002, Berejikian & Tezak 2005). However, due to our poor understanding of the process of fin erosion, it is not currently possible to make suggestions as to how to construct systems to reduce it.

If sunburn is a contributory cause, then provision of shading/roofing and the depth of the water will influence the penetration of ultraviolet light. Water depth has been suggested to be important to aggressive nipping – shallow water may restrict attacks which are generally orientated downwards (Abbott & Dill 1985, Miller *et al*. 1995). If abrasion is a cause of erosion, then the surface texture used to construct the rearing unit will affect fin erosion. The presence and surface of objects in the environment other than tank walls may also potentially abrade fins (Turnbull *et al*. 1998). The relative dimensions of the unit will affect the probability of contact with surface by influencing the volume to surface area ratio. There is however, some doubt as to the role of surface abrasion (see section above entitled ‘Abrasion – tank surfaces and fish’). Alternative hypotheses are that differences in fin erosion are due to concurrent changes in fish orientation, distribution or aggressive behaviour, water quality, and even improved nutrition via within-unit production of additional invertebrate prey (Arndt *et al*. 2001).

Water current

System design will affect the water flow speed. It has been demonstrated experimentally that increasing the water current up to 2 body lengths per second decreases fin damage in Arctic charr and Atlantic salmon (reviewed by Jobling *et al*. 1993). It is thought that this effect is mediated via changes in behaviour – a water flow induces swimming behaviour and reduces aggressive nipping.

Feeding schedule – ration and distribution

There are conflicting reports as to whether feeding rate influences fin erosion. Early research into fin erosion suggested that feeding to satiation would reduce or eliminate fin erosion (Larmoyeux & Piper 1971). Moutou *et al*. (1998) fed

experimental groups of trout differing rations (0.25 to 1.5% body weight per day) and found that lower rations did indeed increase erosion of the D and C fins, which they attributed to increased aggressive behaviour (nipping) at low rations. Winfree *et al*. (1998) interpreted their results as showing that periods of food deprivation caused fin erosion to occur at a smaller size in trout. However, other studies have found contradictory results – reducing daily ration or not feeding for periods of up to 10 days had no affect on D fin erosion (Kindschi 1988, Klontz *et al*. 1991).

Winfree *et al*. (1998) suggested that fin damage occurred during aggregations of fish at feeding time. The distribution of food over space (i.e. feeding area) and time (i.e. number of meals) may therefore affect fin erosion, although directed experimental studies are lacking. Demand feeders have been suggested as a means to reduce fin erosion as they theoretically allow fish to feed to appetite over an extended period. However, experimental studies to date have largely failed to show that demand feeders reduce fin erosion (Kindschi 1984, Wagner *et al*. 1995, 1996a).

Fish species and size composition

The species and size composition of a stock of fish may affect fin erosion by altering levels of aggressive nipping. Reduced dorsal fin damage has been reported in Atlantic salmon held together with Arctic charr, compared to fish held in monoculture (Holm 1989, Nortvedt & Holm 1990). The effect of size heterogeneity within a group of fish on fin erosion has not been examined. However, recent work suggests that aggressive acts (chases and nips) in Atlantic salmon are reduced in the presence of a few large individuals (Adams *et al*. 2000).

Genetics

Although there is some evidence for a genetic influence on fin erosion, the situation is unclear. Bosakowski & Wagner (1994a) noted that fin erosion was worse in cutthroat trout than rainbow trout, which they attributed to domestication of stocks of the latter species. However, Bosakowski & Wagner (1994b) did not find such an effect, and they even found the reverse in a subsequent study (Bosakowski & Wagner 1995). Kindschi *et al*. (1991c) found little difference between wild and domesticated strains of rainbow trout. Rainbow trout are considered to be less susceptible to fin erosion than the anadromous form, steelhead trout (Winfree *et al*. 1998). Bosakowski & Wagner (1994b) also found that the Fish Lake–DeSmet strain of rainbow trout experienced greater fin erosion than other strains; although they did state that as assessment was based on fin size, the possibility of genetic differences in fin length could not be dismissed. One promising lead is that Wagner *et al*. (1996c) found that albino rainbow trout experienced less fin erosion than normally pigmented fish, which they attributed to behavioural differences.

It is currently unclear or unknown whether manipulation of ploidy level (i.e. diploid or triploid) or the production of single sex (all female) groups of fish affects fin erosion (Carter *et al*. 1994).

Temperature

Temperature is likely to affect the causes of fin erosion and hence rate of damage, as well as affecting the healing and hyperplasic responses of damaged fins (see Turnbull *et al*. 1996). Temperature has been shown to affect the severity of fin erosion, although a common trend is not apparent. Winfree *et al*. (1998) compared similar sized trout reared at 10°C and 15°C, and found that the fish from the lower temperature had less fin erosion. In contrast, Schneider & Nicholson (1980) found that fin erosion in Atlantic salmon (attributed to bacterial infection) increased as water temperature decreased. Wagner *et al*. (1998) found no clear temperature-related differences in fin erosion in cutthroat trout. Udomkusonsri & Noga (2005) noted that fin ulceration is often associated with lower water temperatures.

Physiological status

Mork *et al*. (1989) suggested that epidermal structure and mucus production change in salmonids in relation to reproductive status, and that this may then affect fin erosion by affecting damage and healing. They found that mature males showed a lower prevalence of, and better recovery from, fin erosion than immature males. There was also a suggestion that aggressive behaviour, and hence incidence of damage, may be altered by changes in reproductive status.

Can fin erosion be used as a welfare indicator?

Fin tissue is capable of healing and regeneration, which may reflect an evolutionary response to damage under natural conditions. However, as natural damage to the fins of healthy wild fish has not been documented in the literature it can be assumed to be very limited in extent.

Fin erosion does, however, occur in wild fish exposed to organic and inorganic pollution (sewage, pulp mill, industrial – heavy metals, hydrocarbons, effluents) in a wide variety of freshwater and marine species and is associated with other skin lesions and ulcers (e.g. Minchew & Yarbrough 1977, Westernhagen *et al*. 1980, Lindesjöö & Thulin 1990, 1994, Wiklund & Bylund 1996, reviewed by Latremouille 2003). As the skin covering the fins is metabolically active and interfaces with the environment, fin erosion is commonly used as a biomarker for polluted or stressful environments (Iger *et al*. 1995, Udomkusonsri *et al*. 2004).

FAWC (1996) called for the development of methods to objectively assess the welfare of farmed fish. Methods to assess animal welfare on farms can be

divided into two categories, either animal-based (representing the response to the environment) or environment-based parameters based on perceived requirements (Mollenhorst *et al.* 2005). Fin erosion is considered to be a valid animal-based parameter reflecting production techniques (Barrows & Lellis 1999). Although the causes are poorly known, fin state does represent an integrated marker for the quality of the physical, chemical, bacteriological, nutritional, social and behavioural environments. The state of the different fins may even be of use in differentiating different aspects of the environmental quality, e.g. the dorsal fin has been proposed as an indicator of the strength of dominance hierarchies and aggressive behaviour (Moutou *et al.* 1998, MacLean *et al.* 2000).

Lymbery (2002) made a direct link between fin erosion and poor welfare. Whether fin erosion does actually cause suffering is unknown, however both actively eroding and healed (but not regenerated) fins can potentially impact on welfare (see above section: 'Is fin erosion a welfare issue?'). It is therefore reasonable to include fin condition in fish welfare assessments (e.g. Turnbull *et al.* 2005). To assess current environmental quality from single point samples, it will be important to differentiate active from healed erosion, as fin size reflects both historical and current conditions.

Conclusions

Some, but by no means all, farmed fish species are susceptible to fin erosion. Salmonids seem to be particularly susceptible, and the condition has been best studied in this group. However, despite a significant body of research, it remains a poorly defined condition and the process is poorly understood. To improve understanding, it is recommended that attempts are made to differentiate fin erosion from fin rot, and that a common terminology is adopted. The term *fin erosion*, which implies physical abrasion, may prove to be an inappropriate descriptor, but common usage does oblige its continued use for now. Due to the number of causes and factors involved, the fin erosion process is likely to differ between studies and between fins. Fin erosion therefore probably does not describe a single condition, but relates to a series of related conditions on a continuum that also encompasses bacterial fin rot. Further classification of the appearance and state of the damage (e.g. Turnbull *et al.* 1996, Bodammer 2000), rather than simple assessments of size, may shed light in future studies. It would also be useful to have a common standard method for evaluating fin damage, to enable interstudy comparisons.

We do not know what it means to be a fish suffering from fin erosion. Despite this knowledge gap, there is sufficient information to justify fin erosion as a fish welfare issue. Active erosion represents injury with the associated potential for nociception, osmotic disturbance and infection. Previously eroded but healed fins may also compromise welfare if size and form alter function and performance. Fin state can therefore be used to indicate welfare status and should be incorporated into fish quality schemes. Fin erosion can justifiably be highlighted as a significant welfare

concern due to its seemingly high prevalence. Further information is required on the relative importance of active and healed erosion, and the impact of various levels of erosion, with separate consideration of the different fins as their functions differ. Ultimately, an acceptable level of fin erosion needs to be determined as zero tolerance is unlikely to represent a pragmatic target.

The aetiology of fin erosion remains poorly understood due to its complexity. Separate consideration of the different fins and examination of inter-individual variability is merited in future studies. It is widely believed that physical damage is required to initiate the erosion process. There is strong evidence that aggressive nipping is a primary factor in the erosion of the D (and possibly P and C) fins. However, there is a lack of observational data for causes of damage to the other fins due to the inherent difficulties of documenting low frequency acts and then confirming individual physical damage in large populations. The roles of accidental nipping, handling, and sunburn appear worthy of further study. Water quality, infection, nutrition and stress are likely to be secondary factors, but again further definitive research is needed to comprehend their impact on the processes of fin damage, healing and regeneration. Understanding has been hampered by the fact that different studies frequently generate conflicting results which presumably reflects the particular nature of the fin erosion process. The relevance of the various causes and risk factors still remain to be determined and their significance may well differ between specific situations.

It has long been recognised that management practices should be developed to reduce levels of fin erosion (Moring 1982). Once the causal and contributory factors for fin erosion are understood, the severity of fin erosion on farms can potentially be reduced. However, a practical solution has remained elusive. Fin erosion has continued in the US despite the significant effort of scientists and hatchery managers over the last three decades to identify and control risk factors (Winfree *et al*. 1998, Lellis & Barrows 2000). Nevertheless, as fish can be reared with perfect fins (Kindschi *et al*. 1991b), alleviation of fin erosion is thought to be a resolvable research challenge. The fact that the severity of erosion differs between batches of fish and between farms (Bosakowski & Wagner 1994a, St-Hilaire *et al*. 2006) indicates that husbandry and management practices do influence fin erosion, and it can therefore be reduced.

Reduction in fin erosion may involve simple changes to husbandry procedures, e.g. use of knot-free nets to reduce handling damage, shading to prevent sunburn, greater distribution of food to reduce crowding at feeding. Other more complex methods will require more informed research, e.g. the use of appropriate mesh sizes, rearing of less susceptible strains, changes to system construction and design, and modifications to behaviour. As the strongest evidence exists for fin nipping being a primary cause of fin erosion, altering the social environment could be the most significant method to reduce fin erosion (Turnbull *et al*. 1998). Reduction of fin erosion should also be considered in selective breeding programmes alongside the other commonly addressed factors such as growth rate, food conversion ratio (FCR),

flesh pigmentation, and disease resistance. There is a significant body of evidence that the nutritional quality of diets affects fin erosion. Feed manufacturers should be encouraged to take responsibility for development of diets to reduce fin erosion. Given the continuing impetus to substitute fishmeal and oil, future diet composition trials should include assessments of fin erosion (alongside susceptibility to other diseases), and not just focus on growth rates, FCR and cost. The integration of the various strategies into a practical solution to reduce fin erosion is a challenge, but may ameliorate the apparent effects of intensification and thereby increase public acceptance (Lellis & Barrows 2000). Whilst awaiting the outcome of research, farmers should be encouraged to use their own experience to develop and implement local strategies to reduce fin erosion.

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Chapter 10
The Influences of Water Quality on the Welfare of Farmed Rainbow Trout: a Review

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Introduction

For the welfare of farmed fish, the quality of the water is central. It is a primary environmental consideration, with the potential to markedly affect health. Fish exist in intimate contact with the water through the huge surface area of the gills and skin, and it is widely acknowledged that fish are vulnerable to inappropriate water quality. The water provides fish with the oxygen required to survive, dilutes and removes potentially toxic metabolites, as well as providing support against gravity.

Inappropriate levels of water quality parameters affect physiology, growth rate and efficiency, cause pathological changes and organ damage and, in severe cases, cause mortality. The sublethal effects of poor water quality are also commonly linked to increased disease susceptibility, although scientific evidence for direct relationships is lacking. At present, there is insufficient information to conclude if poor water quality has an adverse effect on the welfare of farmed trout.

Salmonids are recognised as being less tolerant of poor water quality, e.g. low oxygen (Wedemeyer 1996) and ammonia (Haywood 1983), than those species that have evolved to inhabit warmer, slower flowing and static waters. Inadequate water quality, as will be illustrated, has a direct impact on fish health, causing either chronic or acute effects. Although frequently considered as a complementary issue to welfare, health is in fact a central tenet of welfare. Inadequate water quality may also have an indirect effect on health by increasing susceptibility to disease.

It is important to consider water quality in terms of both the characteristics of the local catchment supply and the influence of farm management practices. On this basis, water quality parameters can be separated into three categories. The first category reflects the parameters that are largely affected by the biological loading and water treatment systems applied by the farmer and are therefore largely under

their control, i.e. oxygen, ammonia, carbon dioxide, nitrite. The second category includes those parameters that relate to the local catchment water chemistry and are therefore largely outside the control of the farm manager, i.e. acidity, alkalinity, hardness, temperature, conductivity, heavy metal concentration. The third category includes those parameters that can reflect the characteristics of both the intake water and farm management practices, i.e. nitrate, suspended solids, supersaturation. These parameters are discussed in turn below in relation to physico-chemistry, effects on the fish and the practicalities of measurement. Discussion does not include those water quality parameters that may occur sporadically, originating either within the farm (e.g. disinfectants and chemotherapeutants such as ozone and salt), or originating outside the farm (e.g. pesticide or pollutants).

Dissolved oxygen

Dissolved oxygen (DO) is the primary water quality consideration for any salmonid farmer. Oxygen passively diffuses into water from the atmosphere, and the maximum amount that will dissolve depends upon a number of variables including temperature, salinity and altitude. Fish extract oxygen from the water by passive diffusion through the gills. An adequate concentration of DO in the water is required to facilitate the passive diffusion down a concentration gradient from the water into the blood (Colt & Tomasso 2001). If DO concentrations fall below the requirements of the fish, then fish cannot convert energy as efficiently into a usable form, resulting in reduced growth rate, food conversion efficiency and swimming ability (Jones 1971). The opercular ventilation rate increases as DO concentrations decrease, and fish may show a gasping response (Wedemeyer 1996). It has been reported that salmonids show a behavioural avoidance of low oxygen levels (Levy *et al.* 1989) and there are observations that the distribution of fish changes, with fish moving towards the surface or water inflow where DO concentrations are higher (Wedemeyer 1996). There is a lack of information on the effects of a reduced DO concentration on relevant physiological measures of red blood cells (e.g. haemoglobin concentration, cell count, haematocrit). However, when DO approaches lethal levels, effects such as anorexia, respiratory distress and tissue hypoxia precede unconsciousness and death (Wedemeyer 1996).

Existing recommendations

The DO requirements for rainbow trout (*Oncorhynchus mykiss*) have been well studied (Liao 1971, see Smart 1981 for a review). A minimum DO concentration of 5–6 mg/L is frequently recommended for the health of rainbow trout (Smart 1981, Colt & Tomasso 2001). This figure is widely accepted within the industry based upon experience (e.g. Anon 2001). Colt & Tomasso (2001) stated that there are some basic points when considering a minimum DO level, i.e.:

Table 10.1 Solubility of oxygen in freshwater equilibrium with air at 101.325 kPa mg/L (Anon 1980) and minimum recommended DO concentrations for coldwater fish in aquaculture (from Wedemeyer 1996).

Temperature °C	Oxygen solubility, i.e. 100% saturation mg/L	Minimum DO required	
		mg/L	% saturation
5	12.8	9.1	71
10	11.3	8.8	78
15	10.1	8.3	81
20	9.1	7.8	85

- Fish of a given species and size require more oxygen in warmer water than in cooler water, due to their increased metabolic rate in warmer water.
- Fish require a greater amount of oxygen after feeding than before, again due to an increased metabolic rate and the specific oxygen demand.
- Oxygen consumption is proportional to the size and number of fish in a given system.
- Smaller fish use more oxygen per unit weight than larger fish.
- Fish require more oxygen if they have impaired gill function, are exposed to stressors, or if their oxygen-carrying capacity is impaired.

Wedemeyer (1996) suggested that 5–6 mg/L is too low as there is no safety margin for temporary increases in DO requirements due to increased swimming activity, overfeeding and CO_2 increases. As higher water temperatures cause an increase in the metabolic rate and oxygen demand of fish, farmers may encounter problems during summer seasons when the capacity of the water to hold oxygen is reduced. In recognition of this, Wedemeyer (1996) suggests minimum oxygen levels as shown in Table 10.1 to promote good health and physiological condition in the fish stock.

Therefore, even with a parameter as fundamental as dissolved oxygen, there is disagreement regarding a minimum concentration for rainbow trout culture, with recommendations ranging from 5–9 mg/L, depending on the temperature.

Hyperoxia

Oxygenation (i.e. the use of pure oxygen supplementation) is increasingly being used to raise the carrying capacity of intensive fish culture systems (Colt & Watten 1988, Warrer-Hansen 2003). Very little is known about the potential effects of hyperoxia (DO levels $>$ 100% saturation) on fish welfare. Some physiological effects of hyperoxia have been recorded on erythrocyte size and numbers but this was not associated with effects on growth or mortality (Ritola *et al*. 2002). The physical effects of gas supersaturation are discussed elsewhere, but it should be stated that supersaturation is considered to be a less significant problem for oxygen

than for nitrogen, and recommendations for maximum dissolved oxygen levels could not be found in the literature.

Ammonia

The literature relating to ammonia and its toxic effects on fishes is vast, however it is often contradictory and confusing. Several lethal values of ammonia have been reported for rainbow trout, along with many 'safe' levels. In his extensive review of ammonia in aquaculture, Meade (1985) suggests that the reasons for the contradictions in the literature are due to fluctuating ammonia levels caused by variations in diurnal ammonia excretion rates, making predictions about ammonia toxicity difficult, and that the effects of ammonia cannot be predicted based on the concentrations of un-ionised ammonia alone (see below).

Sources

Ammonia is a substance toxic to all vertebrates and is found in the aquatic environment. Sources of ammonia are: excretion by plants and animals; microbial decomposition of organic matter; volcanic emissions; and anthropogenic origins such as the release of fertilizers and industrial emissions (Randall & Tsui 2002).

In aquaculture practices, while ammonia may be present in incoming waters, most of the ammonia found in a fish farm is produced by the fish. Ammonia is the primary waste metabolite produced by fish from the catabolism of protein contained within the feed. The ammonia is excreted from the fish via the gills (Evans *et al.* 2005). Ammonia can also come from the decomposition of uneaten food, although this is considered a relatively minor source (Hinshaw & Fornshell 2002).

Terminology and chemistry

In the aquatic environment, ammonia exists in two forms in equilibrium: as un-ionised ammonia, NH_3, and as ionised ammonium, NH_4^+:

$$NH_3 + H^+ + OH^{[-]-} [\longleftrightarrow] NH_4^+ + OH^{[-]-}$$

Thus, total ammonia concentration is the sum of the concentrations of un-ionised ammonia and ionised ammonium:

$$\text{Total ammonia} = NH_3 + NH_4^+$$

Methods for the measurement of ammonia do not differentiate between the two forms, the proportions of which vary depending upon the position of the equilibrium. The customary UK practice is therefore to express total ammonia concentration as just the amount of nitrogen present – i.e. total ammonia nitrogen (TAN) – rather than trying to include the variable hydrogen component (Anon 1981). The

un-ionised ammonia fraction is referred to as NH_3-N and the ionised ammonium as NH_4^+-N.

The equilibrium between the NH_3 and NH_4^+ varies in relation to the various factors, most significantly the concentration of hydrogen ions (i.e. pH) and temperature. The ionisation constant, pK_a is temperature dependant and can be estimated from temperature according to the following equation:

$$pK_a = 10.055\ [-] - 0.0325(\text{Temp}^\circ\text{C})$$

The percentage of NH_3 can be calculated by entering the pH and pK_a values into the following equation (Wedemeyer 1996):

$$\%NH_3 = 100/(1 + \text{antilog}(pK_a[-] - \text{pH})$$

The NH_3-N is then calculated by multiplying the measured TAN by the $\%NH_3$. Finally, NH_3-N is multiplied by 1.22 to convert to NH_3, thereby correcting for the molecular weight of hydrogen. It is important to recognise that ammonia concentrations are expressed in different ways in different studies, e.g. NH_3, NH_3-N, TAN. Haywood (1983) recommended expressing ammonia concentrations as mg/L NH_3 rather than NH_3-N.

The percentage distribution of each form is therefore highly dependent upon the pH and to a lesser extent the water temperature (Colt & Tomasso 2001). The pK_a value is also affected by ionic strength, pressure and salinity (Colt & Tomasso 2001, Randall & Tsui 2002) although these factors have a minor effect on the distribution of total ammonia forms. The most important factor in determining the distribution of ammonia forms is the pH.

Nature of ammonia toxicity

The distribution of total ammonia between NH_3 and NH_4^+ is important, as the former is considered to be the toxic form to vertebrates, while the ammonium ion is considered to be essentially non-toxic at the levels experienced in aquaculture systems. Most biological membranes are permeable to un-ionised ammonia and relatively impermeable to ionised ammonium (Randall & Tsui 2002). Therefore, in fish, ammonia in the external medium either induces retention of endogenous ammonia in the fish, or the exogenous ammonia enters via the gills by passive diffusion down a concentration gradient (Haywood 1983). However, several authors have questioned the opinion that only un-ionised ammonia is toxic, suggesting that ammonium ions also contribute to the toxicity (Tomasso 1994, Linton *et al.* 1998a).

Acute ammonia toxicity affects the central nervous system of fish (Randall & Tsui 2002), and manifests as a neurological disorder (Haywood 1983). While the exact nature of ammonia toxicity is not known in fish, it appears that ammonia interferes with physiological processes that eventually result in death of cells in the brain. Another theory is that excessive ammonia depolarises muscle fibres and neurons, again leading to cell death (Randall & Tsui 2002).

A suggested detoxification mechanism in fish is that ammonia in the blood is converted into glutamine through the action of glutamine synthetase, an enzyme that is found to be up-regulated during exposure to ammonia (Wicks & Randall 2002a). It is also thought that fish can, to some extent, convert ammonia to urea (Haywood 1983).

Acute toxicity levels

Many studies have been conducted into the acute toxicity of ammonia to rainbow trout. Most studies have investigated the LC_{50}, or the median concentration of ammonia required to kill 50% of the experimental fish within a given period of time, usually 96 hours.

In a series of 81 experiments, Thurston & Russo (1983) found that the 96h-LC_{50} for rainbow trout ranged from 0.16 mg/L NH_3-N to 1.1 mg/L NH_3-N. All experiments were conducted in similar water chemistry conditions and fish were from the same strain. Differences in acute toxicity tolerances were found to be due to different life stages of the test fish. Meade (1985) quoted a 96h-LC_{50} of 0.32 mg/L NH_3-N for rainbow trout.

Short-term exposures of fish to high concentrations of ammonia result in increased ventilation rate, hyperexcitability, erratic swimming, loss of equilibrium, convulsions and death (Smart 1976, 1981, Haywood 1983, Russo & Thurston 1991).

About acute toxicity tests

Acute toxicity tests are used as indicators of concentrations of toxicants that will have an immediate effect on organisms, and are employed in drawing up standards for the control of ammonia concentrations in aquatic systems. In the absence of reliable chronic toxicity test results, a general rule of thumb for ‘safe’ levels for organisms is to use 10% of the 96h-LC_{50} values for maximum limits. Such methodology has led to a suggested maximal level of 0.02 mg/L (Haywood 1983). However, there are several considerations that must be taken into account with regard to toxicity tests. In order to standardise the tests as far as possible, the US Environmental Protection Agency states that acute toxicity studies with ammonia should adhere to the following criteria (Randall & Tsui 2002): exposure of organisms to ammonia should be under static conditions, with the test organisms starved, rested and unstressed. While the rationale behind this allows comparisons to be made between tests, the test conditions only bear limited resemblance to conditions that farmed fish would encounter and therefore the relevance of such tests are questionable.

Chronic effects

Reported effects of chronic exposure to ammonia in the rainbow trout include gill damage (swelling, mucus production, epithelial lifting, hyperplasia, break down of the pillar cell structure of the secondary lamellae, fusion of gill lamellae), ion

imbalances, impaired liver function, impaired renal function, decreased food intake, growth and food conversion, and increased fin erosion (Larmoyeux & Piper 1973, Smith & Piper 1975, Smart 1976, Alabaster & Lloyd 1982, Haywood 1983, Thurston *et al*. 1984, Tomasso 1994, Twitchen & Eddy 1994). In an extensive study into the chronic effects of ammonia on rainbow trout lasting five years and three generations of fish, Thurston *et al.* (1984) found evidence of gill and kidney damage at constant ammonia concentrations up to 0.07 mg/L NH_3 (0.06 mg/L NH_3-N), although there was no evidence that growth or fecundity was affected. However, Daoust & Ferguson (1984) could find no evidence of gill damage in rainbow trout exposed to ammonia concentrations up to 0.4 mg/L NH_3 for 90 days. This led Meade (1985) to conclude that gill damage is probably not caused by ammonia toxicity, proposing a hypothesis that other metabolites and their interactions with water chemistry are possibly involved. With regard to the findings from Larmoyeux & Piper (1973) and Smith & Piper (1975), it should be noted that in both cases the dissolved oxygen concentration was substantially less than saturation and was possibly a factor in the findings of gill damage. It has also been suggested that while the gills may be primarily affected by the external concentration of NH_3 in the water, the internal physiology is affected by the total ammonia concentration (Haywood 1983).

Factors affecting ammonia toxicity

(1) *Dissolved oxygen*. Many researchers have observed that the toxicity of ammonia increases with decreasing DO concentrations (see Russo & Thurston 1991 for a review). Thurston *et al.* (1981a) conducted acute toxicity experiments over a range of DO concentrations and found that tolerance to ammonia decreased with decreasing DO, as shown in Figure 10.1.

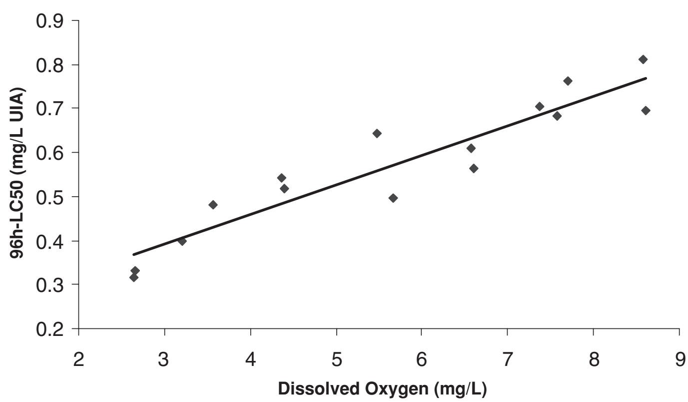

Figure 10.1 Effect of dissolved oxygen on 96h-LC_{50} ammonia toxicity to rainbow trout (from Thurston *et al*. 1981a).

The 96h-LC_{50} for ammonia toxicity for rainbow trout fell by around 30% between DO concentrations of 8.5 and 5 mg/L. As previously discussed, a minimum recommendation for DO concentration is 5 mg/L. Whilst this figure may be adequate to maintain fish health when other water quality parameters are satisfactory, the literature demonstrates that if ammonia concentrations increase, then DO concentrations that were previously believed to be adequate may not be so.

(2) *pH*. The water pH affects the toxicity of ammonia by altering the distribution ratio of the total ammonia forms, as discussed in the section on the terminology and chemistry of ammonia, with an increase in pH resulting in an increase in the fraction of un-ionised ammonia. However, independent of the effect of pH on the equilibrium of ammonia species, Russo & Thurston (1991) found that the 96h-LC_{50} value decreased with decreasing pH over a range of 9 to 6.5. As the lower pH figure is not considered to be toxic, it is possible that the toxic effects were due to the increasing concentration of ammonium ions (NH_4^+) (Tomasso 1994, Linton *et al*. 1998a).

(3) *Temperature*. The effects of temperature on the toxicity of ammonia are not clear; apart from the effect temperature has on the distribution of ammonia forms. Thurston & Russo (1983) observed a decrease in acute ammonia toxicity to rainbow trout as temperature increased over the range 12–19°C. However, some studies noted the reverse of this, or no effect due to temperature (Meade 1985).

(4) *Acclimation*. There is some evidence that prior exposure of rainbow trout to sublethal levels of ammonia increases their tolerance to environmental ammonia (Daoust & Ferguson 1984, Meade 1985, Russo & Thurston 1991). However, Linton *et al.* (1998a) did not find any evidence of acclimation to ammonia, although they suggest that the very low levels of ammonia used during attempted acclimation were not sufficient to trigger an acclimation response. It is suggested that acclimation can occur due to up-regulation of the enzymes involved in detoxification of ammonia (Randall & Tsui 2002).

(5) *Fluctuating ammonia levels*. It has long been recognised that within culture systems, environmental ammonia levels fluctuate hourly due to variability in ammonia excretion levels (Smith & Piper 1975). Thurston *et al.* (1981b) reported that test fish tolerated constant concentrations of ammonia better than fluctuating levels. Given that fluctuating ammonia levels present a more realistic scenario in fish culture conditions, this finding brings into dispute all findings from ammonia toxicity tests where constant ammonia concentrations are used.

(6) *Exercise*. Shingles *et al.* (2001), Wicks *et al.* (2002) and McKenzie *et al.* (2003), reported that swimming increases the toxicity of ammonia to rainbow trout and that increasing levels of environmental ammonia decrease swimming ability. Wicks *et al.* (2002) found that the 96h- LC_{50} was 32 mg/L TAN (around 0.08 mg/L NH_3-N) for exercised fish compared to 207 mg/L

TAN (0.52 mg/L NH_3-N) for rested fish. This figure is significantly lower than other 96h-LC_{50} values (see section on acute toxicity levels).

(7) *Feeding/fasting*. Within fish culture conditions, a primary source of ammonia is the metabolism of the fish. Therefore, it is unavoidable that feeding will have an impact on ammonia levels. There is also evidence that feeding affects the toxicity of ammonia – fed fish are less susceptible to environmental ammonia than unfed fish (Randall & Tsui 2002). This is thought to be due to a more efficient detoxification system in the fed fish. Wicks & Randall (2002b) report that fed fish can tolerate internal plasma ammonia levels on a par with lethal environmental concentrations, which is thought to be due to activation of the ammonia detoxification system (Wicks & Randall 2002a).

(8) *Stress*. There is some evidence that stress increases the toxicity of ammonia to fish (Randall & Tsui 2002), but this is not conclusive. Randall & Tsui (2002) also suggest that fish that are repeatedly stressed up-regulate the ammonia detoxification system, which may afford some protection against ammonia toxicity.

(9) *Ionic strength of water*. The ionic strength of water (as measured by dissolved solids) affects the equilibrium of the two forms of ammonia, albeit to a much lesser extent than pH and temperature (Messer *et al.* 1984). Meade (1985) reported that in fresh water, ammonia toxicity increases as the ionic strength of the water moves away from the ionic strength of the blood of the fish, which is roughly a third of the strength of sea water (Fevolden *et al.* 2003). Ammonia has a diuretic effect on rainbow trout, and therefore fish must replace the ions that are lost in the urine (Lloyd & Swift 1976); increasing the salinity of the water reduces the osmoregulatory cost of increased ventilation that is incurred as a result of exposure to ammonia. Furthermore, some authors have suggested that ammonia is actively transported out of the body through a NH_4^+/Na^+ pump, therefore higher concentrations of Na^+ in the water will enhance this, reducing the concentration of ammonia in the fish and relieving some of the effects of toxicity (Soderberg & Meade 1992). However, some authors dispute the existence of the NH_4^+/Na^+ pump (Wilson *et al.* 1994), asserting that all ammonia excretion in freshwater rainbow trout is through passive diffusion. Calcium and other divalent cations in the water (e.g. Mg^{2+}) are known to decrease the gill membrane permeability and can increase sodium influx, which could also reduce the toxicity of ammonia (Soderberg & Meade 1992). In one study, an increase in the calcium ion concentration was shown to ameliorate ammonia toxicity in rainbow trout (Wicks *et al.* 2002).

(10) *Life stage and size*. In their series of acute toxicity experiments, Thurston & Russo (1983) found that tolerance to ammonia toxicity increased as fish developed from the larval stage, to a maximum tolerance as juveniles (around 1–4 g), following which tolerance to ammonia decreased. In his review of ammonia, Meade (1985) reports that tolerance of rainbow trout was up to 50 times greater in fish that had not fully absorbed the yolk than in adult trout.

Existing recommended levels

From the literature, there is widespread disagreement regarding safe levels of ammonia in culture systems for rainbow trout. Hampson (1976) recommends a maximum limit of 0.3 mg/L NH_3-N, while Wedemeyer (1996) recommends no more than 0.02 mg/L NH_3. Following their 6-month trial on rainbow trout, Smith & Piper (1975) recommended a maximum ammonia concentration of 0.0125 mg/L NH_3, which was the 'no observable effect concentration' for growth. However, it should be noted that the dissolved oxygen in that experiment was low, with an average of around 6 mg/L. The recommended maximum of 0.0125 mg/L NH_3-N was nonetheless echoed by Westers and Pratt (1977) and Soderberg *et al.* (1983). Following a review of various studies, Haywood (1983) recommended maximum levels of only 0.002 mg/L for salmonids and added that total ammonia levels should also be below 1 mg/L to account for uncertainty on the toxic action of ionised ammonia.

Meade (1985) contended that differences between different culture systems and water chemistry make recommending a maximum 'safe' level of ammonia for rainbow trout inappropriate, as ammonia concentrations in one system may affect fish health while the same concentration in another system may have no effect. Klontz (1991) differentiated between intermittent and constant concentrations of ammonia, recommending maxima of 0.05 mg/L and 0.03 mg/L NH_3 respectively.

Positive effect of ammonia

While ammonia is recognised as a toxicant and is detrimental to the health of fish, there is some evidence that low concentrations of ammonia can stimulate growth. Studies by Linton *et al.* (1998a) showed increased growth at low TAN levels of 1.96 mg/L (around 0.035 mg/L NH_3-N), which agreed with earlier work at the same laboratory (Linton *et al.* 1997, 1998b) and subsequent work carried out by Wood (2004). Wood (2004) postulated that low ambient levels of ammonia either stimulate ammonia incorporation into amino acids and protein synthesis and/or reduce metabolic costs, as growth was improved without an alteration in food consumption by the fish. This, however, conflicts with prior studies where growth was suppressed at ammonia concentrations as low as 0.002 mg/L NH_3-N (Russo & Thurston 1991).

Nitrite

Nitrite, $NO_2^{[-]-}$, is formed from the oxidation of ammonium (NH_4^+) in the aquatic environment. Nitrifying bacteria, *Nitrosomonas* spp., oxidise ammonium into nitrite. The bacteria *Nitrobacter* spp. then convert nitrite into nitrate, $NO_3^{[-]-}$ (Lewis & Morris 1986).

Nitrite can be found in high concentrations naturally, such as in deep stratified lakes in the hypolimnetic layer (Boyd 1990). Within aquaculture systems, the primary source of nitrite is the oxidation of ammonium produced by the fish. Nitrite concentrations may increase if oxidation rates of ammonia exceed oxidation rates of nitrite (Colt & Tomasso 2001), or if the oxidation process is inhibited, e.g. by ammonia (Russo & Thurston 1991). However, in trout farming, nitrite produced within the farm is generally not problematic in flow-through systems which constantly flush and remove organic wastes and which predominate in the industry. An exception to this is in malfunctioning recirculation systems when biological filtration is relied upon to maintain water quality. However, the main sources of high nitrite concentrations are anthropogenic in origin, such as from sewage effluents and agricultural drainage (Wedemeyer 1996); these pose the main nitrite threat to trout farming by affecting the initial water intake.

In freshwater fish, nitrite enters through the gills. Nitrite ions are actively taken up through the chloride cells and can be pumped in against a concentration gradient (Jensen 2003), which can result in blood plasma concentrations of nitrite up to ten times that of the ambient water concentration (Eddy *et al*. 1983).

Nitrite is toxic to fish as it diffuses from the blood plasma into the red blood cells, where it oxidises the Fe^{2+} in haemoglobin (Hb) to the Fe^{3+} oxidation state, converting haemoglobin into methaemoglobin (metHb). MetHb lacks the capacity to bind to oxygen, therefore the oxygen transport system in the fish is disabled resulting in hypoxia. The build up of MetHb is known as methaemoglobinaemia, or more commonly, brown blood disease, named after the characteristic colour of blood and gills of chronically nitrite-exposed fish or other animals. MetHb occurs naturally in the blood of fish, typically at levels of 1–3%, however, levels in excess of 10% are detrimental to fish health, and clinical signs have been reported with levels over 25% (Lewis & Morris 1986). Nitrite exposure may also damage the gills (hypertrophy, hyperplasia, epithelial separation) and the thymus (haemorrhage and necrotic lesions) (Wedemeyer 1996). The thymus is located in the gill cavity and is involved in the production of lymphocytes (Bowden *et al.* 2005).

Nitrite-induced metHb is a reversible condition, as the red blood cells of fish contain an enzyme, metHb reductase, that reduces metHb to Hb (Scott & Harrigan 1985). If nitrite levels in the water are reduced before metHb levels become lethal, the fish should fully recover (Jensen 2003).

Aside from the indicative brown blood found in exposed fish, gross signs of methaemoglobinaemia are lethargy as blood levels of metHb approach 70–80%, with disorientation and unresponsiveness reported at levels near 100% (Westin 1974). The lethargy and lack of activity reported in fish with methaemoglobinaemia may well be a behavioural response to cope with the condition, as this reduces their oxygen demand. However, should the fish be startled or forced to become active, they may then die from hypoxia (Huey *et al*. 1980).

Table 10.2 Selected acute nitrite toxicity figures for rainbow trout (from Lewis & Morris 1986).

Nitrite[bond]-N 96h LC_{50} (mg/L)	$Cl^{[-]-}$ (mg/L)	pH	Ca^{2+} (mg/L)	Alkalinity ($CaCO_3$, mg/L)
0.24	0.35	7.9	60	176
3	10	7.7	52	171
8	20	7.7	52	171
11	40	7.7	52	171

Toxicity

96h-LC_{50} values for rainbow trout range from 0.19 to 12.6 mg/L $NO_2^{[-]-}$-N (see reviews by Lewis & Morris 1986, Russo & Thurston 1977, 1991, Russo *et al.* 1981, Eddy & Williams 1994). There are several reasons for this range being over two orders of magnitude, however the primary reason is water chemistry, or more specifically the chloride ion concentration of the water. Nitrite is transported into the fish through chloride cells in the gills, and it appears that the presence of chloride ions in the water compete with the nitrite for transport; as the concentration of chloride ions increases, so the uptake of nitrite decreases. Table 10.2 demonstrates the effect of chloride ions on nitrite toxicity.

Other anions in the water that affect nitrite toxicity are bromide and bicarbonate. Bromide was shown to have a greater effect on nitrite toxicity than chloride (Eddy *et al.* 1983), however as bromide is not typically present in fresh waters, this is of academic interest only. Bicarbonate inhibits the uptake of chloride from water, and appears to have the same effect on nitrite uptake, although it does not affect nitrite toxicity to the same degree as chloride (Lewis & Morris 1986). Sulphate, phosphate and nitrate have also been shown to affect nitrite toxicity (Russo & Thurston 1991). There is some evidence that calcium ions (Ca^{2+}) may increase the inhibitory effects of chloride on nitrite toxicity through its action on the gill membrane (Tomasso 1994).

Low concentrations of dissolved oxygen affect the toxicity of nitrite (Lewis & Morris 1986). As nitrite affects the ability of the blood to transport oxygen, a reduction in ambient water DO concentrations will exacerbate the effect of toxicity. The effect of temperature on the toxicity of nitrite to rainbow trout is not known; however there is inconclusive evidence from studies on the channel catfish (*Ictalurus punctatus*) that higher temperatures can increase nitrite toxicity (Lewis & Morris 1986). With regard to the size of fish, there is some evidence that smaller rainbow trout are more tolerant of nitrite than larger trout (Lewis & Morris 1986).

Aside from studies into the acute lethal effects of nitrite toxicity, there have been very few studies into the long-term chronic effects in rainbow trout. Wedemeyer & Yasutake (1978) exposed rainbow trout to nitrite concentrations up to 0.06 mg/L

$NO_2^{[-]-}$-N for 6 months in soft water with a low chloride content. There were no mortalities, growth was not significantly different between treatments, and only mild methaemaglobinaemia was noted (around 5%). Hypertrophy of the gills was observed, with the most severe cases noted around 4 weeks into the trial; after 7 weeks, hypertrophy was observed less frequently and at the conclusion of the trial no hypertrophy was recorded, indicating that the fish were able to acclimate to the nitrite concentrations.

From their review of the literature, Lewis & Morris (1986) concluded that lethal concentrations over 96 hours and concentrations showing minimal or negligible effects only differ by a few-fold, indicating that if the fish survive the initial exposure, then they can probably acclimate and survive ongoing exposure.

Existing recommendations

The recommended maximum concentration for nitrite is 0.1 mg/L $NO_2^{[-]-}$ (Wedemeyer 1996) ($\equiv$ 0.03mg/L $NO_2^{[-]-}$-N). However, the chloride concentration and to some extent the concentration of other ions in the water, will have a major effect on the toxicity of any nitrite present.

Nitrate

Nitrate is produced from the oxidation of nitrite by the bacteria *Nitrobacter* spp. (Lewis & Morris 1986). The 96h-LC_{50} for salmonids is in the range of 1000–3000 mg/L $NO_3^{[-]-}$-N (Wedemeyer 1996). Nitrate within flow-through aquaculture systems is generally dismissed as not being a threat to the health of older life stages of farmed rainbow trout (Russo & Thurston 1991, Tomasso 1994, Wedemeyer 1996). However, a maximum value of 1 mg/L is suggested by Wedemeyer (1996) as a guideline, as exposure of eggs to nitrate can result in developmental problems. Therefore nitrate exposure via inflow water poses a significant potential threat during the hatchery stages. Nitrate levels in many English waters, both ground and surface waters, are increasing due to agricultural run-off, with concentrations around 50 mg/L being found in a number of areas (Defra 2004).

Carbon dioxide

Carbon dioxide (CO_2) is found naturally in most surface waters at levels of 1–2 mg/L and originates from diffusion from the atmosphere, microbial decomposition of organic matter in sediments and the respiration of micro-organisms, algae and aquatic plants (Wedemeyer 1996). Naturally higher levels of CO_2 can be found in well or spring water. Within aquaculture systems, the primary source of carbon dioxide is through fish metabolism. CO_2 is considered to represent an increasingly important issue as more intensive production technologies, i.e. oxygen injection,

are being introduced (Summerfelt 2002). As a rough guide, for each unit of oxygen that a fish respires, around 1.4 units of carbon dioxide are generated (Westers 2001).

Carbon dioxide reacts with water when it dissolves, forming a mixture of CO_2, carbonic acid (H_2CO_3), bicarbonate ($HCO_3^{[-]-}$) and carbonate ($CO_3^{2[-]-}$) ions:

$$CO_2 + H_2O\,[\longleftrightarrow]\,H_2CO\,[\longleftrightarrow]\,H^+HCO_3 + [\longleftrightarrow]\,H^+CO_2^{[-]-}$$

The percentage distribution of each form is determined mainly by the pH. At a pH less than 5, the dominant form is dissolved CO_2; at pH between 7 and 9, the bicarbonate ion is the dominant form; while at pH 11, the carbonate ion has the greatest percentage. Carbonic acid is only present in water in very small quantities and is generally discounted (Wedemeyer 1996). As CO_2 dissolves, hydrogen ions are released, decreasing the pH of the water, and further increasing the proportion of CO_2 present in the dissolved form (Westers 2001).

Toxicity

Out of the forms of dissolved carbon dioxide in the water, CO_2 and carbonic acid are the toxic forms, while the bicarbonate and carbonate ions are not toxic (Wedemeyer 1996). Carbonic acid is generally discounted as having any influence on toxicity due to the small quantities present ($<1\%$).

Carbon dioxide is toxic to fishes because increases in ambient CO_2 concentrations result in the fish being unable to excrete endogenous carbon dioxide, leading to CO_2 increases in the blood, known as hypercapnia. As a result of this, the blood pH decreases, leading to acidosis, reducing the oxygen carrying capacity of the blood in a process called the Bohr effect. The reduction in blood pH weakens the bond between haemoglobin and oxygen molecules, resulting in the release of oxygen molecules which then passively diffuse into cells that have a low partial pressure of oxygen. This effect has been observed in salmonids at water concentrations of CO_2 of around 20 mg/L (Westers 2001). Danley *et al.* (2001) recorded reduced growth in rainbow trout over a 90-day experiment with CO_2 concentrations up to 45 mg/L, but there was no report of significant mortalities at this level. Clinical signs of carbon dioxide toxicity include moribund fish, gaping mouths, flared operculae, and bright red gill lamellae (Summerfelt 2002).

A well-known effect of CO_2 in conjunction with hard water is nephrocalcinosis (Harrison 1979a, b, Smart 1981, Fikri *et al.* 2000). This chronic degenerative condition of the kidney is characterised by calcareous deposits (Harrison & Richards 1979, Smart *et al*. 1979). The white gritty kidney deposits consist of calcium salts, occur within the ureters on the surface of the kidneys, and the kidneys become swollen, sometimes with fluid-filled cysts (Harrison 1979a). The kidney is a major haemopoeitic organ in fish, and blood haematocrit values and haemoglobin content decrease in affected fish (Yurkowski *et al.* 1985). Severely affected fish become dark in colour, have a swollen abdomen and most of the functional kidney tissue is destroyed (Harrison 1979a, Yurkowski *et al.* 1985).

Nephrocalcinosis occurs when natural CO_2 levels in the water are high and/or when additional oxygenation is used to increase carrying capacity, and the total amount of metabolic CO_2 excreted rises as a result (Harrison 1979b). CO_2 levels of 12 mg/L induce nephrocalcinosis, with higher concentrations increasing the prevalence and severity of the condition (Harrison 1979b, Smart *et al.* 1979). Although CO_2 level is a primary factor in the induction of the condition, a variety of physico-chemical factors associated with water chemistry, diet composition, strain and species of fish are involved in its development (Harrison & Richards 1979, Smart *et al.* 1979). Nephrocalcinosis was highlighted as an issue in farmed UK trout populations in the 1970s, but has received little attention since. Nephrocalcinosis has recently been reported in rainbow trout in Israel and Atlantic salmon smolts in Norway (Fikri *et al.* 2000, Fivelstad *et al.* 2003a). Possible methods to manage the condition include increased dietary magnesium, or avoiding susceptible strains and species or pre-disposed sites (Harrison 1979a, b).

Existing recommendations

Wedemeyer (1996) recommends that CO_2 levels should not exceed 10 mg/L, although Smart (1981) reported that there was no reduction in growth or food conversion ratio at CO_2 levels of 24 mg/L. Heinen *et al.* (1996) recommend safe levels as being between 9–30 mg/L based upon their literature review. Noble & Summerfelt (1996) state that safe levels vary due to other water quality factors (such as DO, pH and alkalinity), which must all be taken into account when considering recommended safe levels for aquaculture.

Suspended solids

The literature pertaining to the effects of suspended solids on fish is surprisingly sparse, considering the potentially severe impacts that high levels of suspended solids can have on aquaculture production. This is likely to be due to the wide variability in the nature of the particulate matter and the different effects that the various forms of suspended solids have on fish. There is however a wealth of literature relating to suspended solids in effluent waters from fish farms (e.g. Beveridge *et al.* 1991, Hinshaw & Fornshell 2002, Tucker *et al.* 2002) and the effects these have on the flora and fauna of receiving waters.

Suspended solids come in a wide variety of materials (clay, volcanic ash, pollen, uneaten food, faeces) in a variety of sizes and shapes (Klontz 1993, Wedemeyer 1996). Solids such as clay and soil sediments occur naturally (Boyd 1990), or through anthropogenic influences such as mining, logging or construction (Colt & Tomasso 2001). Such suspended solids will typically enter the farm in the inflow water. Within fish culture systems, uneaten food, faecal solids (Wedemeyer 1996), microfauna (Chen *et al.* 1994) and build up from biofilters that have broken off in

recirculation systems (Noble & Summerfelt 1996) contribute to the total suspended solids.

Suspended solids are defined as particulate matter within the water with a diameter greater than 1 μm. Solids have organic and inorganic components, with the organic section known as volatile suspended solids (Chen *et al.* 1994). Solids can also be classified as settleable or non-settleable, with the larger settleable solids having a diameter greater than 100 μm. Non-settleable solids tend to be the most problematic in culture systems; mortalities have been reported within an intensive rainbow trout farm associated with suspended solids with a diameter of 5–10 μm (Chen *et al.* 1994). The construction of the culture system and the rate of water flow will influence the amount of suspended solids in the system at any one time. For example, self-cleaning raceways use water velocities in excess of 3 cm/sec to prevent solids such as uneaten food from settling (Wedemeyer 1996).

Suspended solids have been shown to affect fish health by physically abrading or clogging the gills, smothering eggs during incubation, abrading the skin and impairing visual feeding (Alabaster & Lloyd 1982, Wedemeyer 1996). Redding *et al.* (1987) showed that steelhead trout (the anadromous form of rainbow trout) exposed to suspended solids over 400 mg/L, suffered a classic stress response of increased blood cortisol. However, they reported no gill damage despite exposing the test fish to suspended solid concentrations up to 3000 mg/L for up to 8 days. Magor (1988) reported gill damage such as lamellar oedema and telangiectasis (dilation of the capillaries) in coho salmon, *Oncorhynchus kisutch*, exposed to suspended solids as low as 44 mg/L. Alabaster & Lloyd (1982) reported that rainbow trout could survive for a day in suspended solid concentrations of 80 000 mg/L, and that they could survive for 10 months in suspended solid concentrations of 200 mg/L, although the type of solid (material, shape and size) affects the effect on fish.

In addition to the above direct effects of suspended solids, there may be indirect effects on fish health. Organic suspended solids have the potential to increase the biological oxygen demand of the culture system, thereby reducing the dissolved oxygen (Chen *et al.* 1994) and some solids can mineralise to produce ammonia (Liao & Mayo 1974). Some micro-organisms associated with suspended solids produce CO_2 through respiration resulting in a reduction of water pH, and some micro-organisms can be facultative fish pathogens (Noble & Sommerfelt 1996).

Existing recommendations

Due to the potential variability in the size and type of suspended solids, few recommendations for maximum suspended solids exist. The shape, in particular the presence of irregular sharp edges, will affect the degree of abrasive impact (Wedemeyer 1996). Wedemeyer (1996) suggests 80–100 mg/L total suspended solids (TSS) as a guide for a reasonable maximum chronic exposure level, while Chen *et al.* (1994) suggest a maximum of 15 mg/L TSS. Alabaster & Lloyd (1982) state that there is no evidence of effects at concentrations under 25 mg/L.

Gas supersaturation

Supersaturated water has been recognised as a problem for fish culturists for over 100 years (Garton & Nebeker 1977). Supersaturation occurs when the partial pressure of one or more of the gases dissolved in the water becomes greater than the atmospheric pressure. Under normal conditions, the partial pressures of the gases dissolved in water are in equilibrium with the atmospheric gases. However, this balance can be altered by natural means, such as large waterfalls, sudden temperature changes or through anthropogenic influences, such as from large dams (Garton & Nebeker 1977). Within aquaculture systems, supersaturation can be due to a variety of mechanisms: sudden increases in temperature; sudden decreases in pressure (e.g. when ground water comes to the surface via borehole pumping or natural springs); entrapment of air in piped supplies or in spillways of dams; and oxygen injection systems (Doulos & Kindschi 1992, Wedemeyer 1996).

Gas supersaturation becomes a fish health issue when it manifests as gas bubble disease (GBD), which is similar to decompression sickness experienced by scuba divers. The blood and tissues of fish will equalise with the partial pressures of the ambient water, therefore if the ambient water is supersaturated, then the blood and tissues of the fish will also become supersaturated. Bubbles of gas, known as gas embolisms, may then form in the vascular system through a change in venous blood pressure, and are rapidly carried to the skin, mouth and fins (Wedemeyer 1996). Depending on the severity of the condition, tissue necrosis and death may result (McDonough & Hemmingsen 1985) and embolisms in the heart or other vital organs normally cause death (Wedemeyer 1996). Fish may recover if held under greater hydrostatic pressure (i.e. in deeper water) and the pressure gradually reduced, or if the temperature is reduced gradually (Wedemeyer 1996).

Recommendations

Wedemeyer (1996) noted that recommending a maximum figure for supersaturation is difficult; maximum chronic safe exposure limits vary with species, size and environmental conditions (e.g. depth affects hydrostatic pressure). He suggested that for salmonids, supersaturation should be $\leq$103% for hatchery stages and $\leq$105% for on-growing stages.

Acidity

Acidity is the quantitative capacity of water to react with a strong base to a designated pH (APHA 1998). Acidity should be measured by titration with a standard base such as 0.02 N NaOH to the phenolphthalein end point at a pH of 8.3, and expressed as milliequivalents per litre (meq/L) (APHA 1998). However, the process of titration is time consuming and requires specialist equipment, therefore acidity is often expressed as pH, which is a measure of the negative logarithm of the

concentration of hydrogen ions (H^+) present at 25°C. A pH of 7 is considered neutral, <7 acidic, and >7 is alkaline.

Waters can be naturally acidic, however within aquaculture systems, the pH can fall due to respiration and excretion of CO_2 from the fish (see section on carbon dioxide). If the inflow water to the culture system is soft or has low alkalinity, then the decrease in pH could become a problem for the fish, as the water has no buffering capacity to protect against the pH change.

Acidic waters have been shown to reduce the swimming capabilities of rainbow trout (Ye & Randall 1991); to affect the acid–base regulation (McDonald *et al.* 1980) and the regulation of ions (Ye *et al.* 1991); to interfere with the ability of fish to excrete ammonia (Wright & Wood 1985, Randall & Wright 1989), carbon dioxide and to transport oxygen (Randall 1991); and to increase the toxicity of ammonia (see above).

Existing recommendations

Existing recommendations for fish health are for the water to have a pH no less than 6, as above this figure the effects of acidity are negligible (Randall 1991). Aside from the direct effects of acidity on fish, a reduction in the water pH affects other water chemistry parameters, for example the distribution of ammonia forms (see section on ammonia), or the solubility of toxic metals in the water, for example aluminium (Wedemeyer 1996).

Alkalinity

Alkalinity is a measure of the total concentration of alkaline substances dissolved in the water. It is the capacity of water to neutralise hydrogen ions (H^+) and is measured by titration with standardised acids to the methyl end point of pH 4.3 and expressed as milliequivalents/L (meq/L) or mg/L (as calcium carbonate, $CaCO_3$) (APHA 1998). The majority of waters with high alkalinity also have an alkaline pH and a high concentration of total dissolved solids.

As with water hardness (see next section), alkalinity has the potential to provide protection to the water system by buffering against large and sudden pH changes. However, while the properties of alkalinity are usually beneficial, highly alkaline waters can also be problematic for fish, as ammonia excretion and production can be inhibited (Wright & Wood 1985, Wilson *et al.* 1998), which can result in toxic levels of ammonia in the fish (Wedemeyer 1996).

Recommended level

Wedemeyer (1996) provides recommendations for upper and lower limits for alkalinity: >20 mg/L (to provide some capacity for buffering against pH extremes) and <100–150 mg/L (as $CaCO_3$) (to ensure that ammonia excretion is not inhibited).

Table 10.3 Classification of water in terms of hardness, as shown in Wedemeyer (1996).

Soft	< 75 mg/L (as $CaCO_3$)
Moderate	75–150 mg/L
Hard	150–300 mg/L
Very hard	> 300 mg/L

Hardness

Hardness is primarily a measure of the amounts of calcium (Ca^{2+}) and magnesium (Mg^{2+}) salts that are present in the water (APHA 1998). Although other divalent dissolved metals such as iron (Fe), copper (Cu), lead (Pb) and zinc (Zn) also contribute to total water hardness, these elements are usually present in such small quantities that hardness is generally taken as a measurement of calcium and magnesium salts (Wedemeyer 1996).

As a method of classifying water for use in aquaculture, water hardness and alkalinity are probably the most useful measurements for biological systems. As with alkalinity, hardness is also used as a measure of the buffering capacity of the water. Soft water is usually acidic and hard water is usually alkaline. Water can be classified in terms of hardness as shown in Table 10.3 (Wedemeyer 1996).

In fresh water, rainbow trout must regulate the concentration of ions in their blood through active transport of ions from the water through the gills. The regulation and transport of these ions is a vital task to enable the fish to maintain homeostasis, as ions are lost from the blood by diffusion through the gills and through the copious amount of urine produced by freshwater fish. The active transport of ions into the fish requires energy and is carried out against a concentration gradient. In soft water, the concentration gradient is very large (up to 3000 times between blood and water) (Wedemeyer 1996) and can use several percent of the energy provided by the diet. In harder water, the concentration gradient is far less and therefore less energy is required to regulate the blood ionic content (Klontz 1991, Wedemeyer 1996).

Additionally, water hardness is important in aquaculture, as it provides an indication of the calcium and magnesium carbonate buffering capacity of a system, which controls changes in the pH (Howells 1994). Water pH affects the toxicity of various compounds (ammonia, carbon dioxide, heavy metals) and therefore water hardness will influence the effects that these compounds will have on fish through regulation of pH.

Temperature

Temperature is a vitally important physical property of the water in aquaculture systems. The temperature of the water regulates the amount of dissolved oxygen

that a body of water can hold, the rate of decomposition and photosynthesis which will affect the oxygen demand in pond systems, and the ionisation of ammonia (see above) (Colt & Tomasso 2001). Additionally, increasing temperature increases the growth and infectiousness of many fish pathogens (Roberts 1975) and increases the toxicity of many dissolved contaminants (Wedemeyer 1996). All of these factors have the capacity to compromise the health of farmed fish.

As fish are exothermic, increasing the water temperature increases the metabolic rate and hence oxygen consumption. It has been calculated that raising the water temperature from 9°C to 15°C reduces the capacity of water to hold oxygen by 12.8%, while increasing the metabolic rate of a 100 g rainbow trout by 67.5% and increasing ammonia excretion by 98.6%, leads to a 58.8% increase in environmental un-ionised ammonia (Klontz 1993).

Existing recommendations

Optimum temperatures for growth and spawning have been examined for many species important to aquaculture. For the rainbow trout, the optimum temperature range is suggested to be 16–17°C for growth and 10–13°C for spawning (Colt & Tomasso 2001). However, these ranges should only be regarded as guidelines. Wild rainbow trout are exposed to a wide seasonal temperature range characteristic of high latitudes. Temperature optima are primarily determined by the genetic tolerance of the fish to temperature (Wedemeyer 1996), and therefore temperature optima will differ between strains originating from different areas. Other factors that will affect temperature optima are the length of acclimation time, the DO concentration and the ions present in the water (Wedemeyer 1996). Based on avoidance experiments, Neill & Bryan (1991) noted that the specific temperature at which fish displayed avoidance behaviour varies by ±5°C. They also stated that preferred temperatures are size specific, and depend on previous temperature acclimation history. Lethal temperatures have been estimated for rainbow trout at 0°C and 26°C (Wedemeyer 1996); however again these maxima should be treated with caution. The recommended range for salmonid culture is 7–18°C for on-growing and 8–10°C for eggs and fry. Inappropriate rearing temperatures have been associated with a number of deformities in salmonids in both hard tissues (foreshortened maxillae, gill operculum shortening, vertebral abnormalities leading to 'short tails' and 'humpbacks') and soft tissues (swim bladder torsion, missing septum transversum) (Fish Farming International 1999, see Chapter 13).

Conductivity

The conductivity of water is a measure of its ability to convey an electrical current (Boyd 1990), which indicates the ionic activity and content of the water. While different ions have different abilities to conduct electricity, generally the higher the

concentration of ions, the greater the conductivity. Conductivity of fresh waters is usually in the range of 20–1500 μmhos/cm (μS/cm; Boyd 1990) with brackish water and sea water having far greater conductivities due to the large number of ions present.

Conductivity does not directly affect the welfare of fish, however it is a good indicator of the general condition of the water. Taking conductivity measurements can assist in evaluating variations in mineral concentrations in water and can also assist in estimating the total dissolved solids present in water. Mineral concentrations and total dissolved solids have the capacity to affect other water chemistry parameters, such as pH. There are no recommendations for conductivity levels for fish health/welfare, as each body of water will have a range of conductivity levels, however once that range has been established then variations away from that range can indicate that there may be a potential problem.

Heavy metals

Heavy metals that may potentially present fish health problems in aquaculture systems include copper, cadmium, lead and zinc. In addition to natural sources of these elements, heavy metals may be introduced in culture systems through industrial discharges, or from their use in weed control. While these metals are generally only present in surface waters in trace amounts, they can be very toxic to fish, including rainbow trout (Wedemeyer 1996). In soft water, heavy metal ions are highly soluble and highly toxic; however hard, alkaline waters result in precipitation of the metals with carbonates or hydroxides, which reduces their toxicity. Suspended solids may also alleviate the effects of heavy metals as the ions will adsorb on to the particles. High temperatures, low dissolved oxygen and high concentrations of dissolved carbon dioxide increase the toxicity of the metals. Acute exposure by rainbow trout to lethal levels of zinc or copper may not become evident until one or two days after exposure, at which time mortalities in the stock will start to occur. However, water chemistry analysis at that time may be too late to detect heavy metals as being the cause of the mortalities, with only background levels showing (Wedemeyer 1996).

Existing recommendations

For maximum recommended concentrations of some heavy metals, see Table 10.7 in the discussion.

Water flow

The flow of water through a fish culture system greatly influences the water quality in the system, by replenishing dissolved oxygen and flushing out metabolites such as ammonia, nitrite and carbon dioxide. The flow of water can also assist in removing suspended solids. Recommendations have been made for flow rate in relation to

the biomass, i.e. loading rate. However, such recommendations, e.g. 1–4 kg/L/min vary widely; it has been suggested that it is dependent upon temperature and fish size (Anon 2001).

The flow rate of water through a system will affect the speed of the current, which may have a knock-on effect on fish welfare independent of water quality considerations. The relationship between water flow and current speed will be determined by the design of the system, e.g. raceways versus ponds. It has been suggested that a moderate current speed provides exercise, improves physiological performance and growth, and reduces physical damage to the fins through behavioural changes (Jobling *et al.* 1993). Jobling *et al.* (1993) recommended current speeds of 0.75–1.5 body lengths/sec for salmonids.

Discussion

It is clear from this review that numerous different water quality parameters have the potential to have an adverse impact on the health (and hence welfare) of farmed rainbow trout. The various water quality parameters can be classed, albeit subjectively, into tiers of importance with regard to potential impact on the fish:

Tier 1: Oxygen
Tier 2: Ammonia, carbon dioxide, gas supersaturation
Tier 3: Nitrate, suspended solids, temperature
Tier 4: Nitrite, acidity, alkalinity, hardness, conductivity, heavy metals

This ranking is believed to be in line with opinions prevalent within the UK trout industry, reflects the fact that trout are typically farmed in agricultural rather than industrial areas, and that the use of low flow, static or recirculation systems is limited. Although water reuse is common (i.e. units receiving the outflow from upstream units), the flushing rate and lack of biofiltration mean that ammonia is of greater significance than its oxidation products.

Is poor water quality a cause for concern?

Fish may theoretically be exposed to inadequate water quality during routine rearing and/or during sporadic events such as transport, handling, grading, and harvest. However, there is insufficient information available at present to conclude whether poor water quality is affecting the health and welfare of trout currently farmed in the UK. It must be acknowledged that financial considerations in intensive fish farming lead to the temptation to push the carrying capacity of water flow to the limit. Nevertheless, it must be stressed that it is in the farmer's own economic interests to ensure that water quality does not have an impact on production, and farmers use their experience to avoid adverse impacts of water quality deterioration. Growth rate, food conversion efficiency and disease incidence will be sensitive to water quality (see below) and adverse effects will impinge on profit margins.

Monitoring welfare in relation to water quality

Farmers have a duty of care to prevent or minimise the impact of poor environmental conditions on their animals. Quality assurance schemes and legislators also have a responsibility to ensure that fish are not exposed to adverse water quality if this leads to suffering. So how can this be achieved? Animal welfare can be monitored through either environment-based (i.e. requirements for good welfare) or animal-based (the responses to the environment) parameters (Mollenhurst *et al.* 2005).

Environment-based parameters

Prescription of water quality limits is an attractive option for safeguarding fish welfare. However, there are two main problems with such an approach, namely the standardisation of measurement and the setting of appropriate limits.

If water quality limits were introduced, farmers would need the capacity to self-monitor the parameters, and such measurement would have to be standardised. Standardisation would have to take into account:

(1) The timing and frequency of sampling. These would need to be prescribed, and be appropriate for the anticipated fluctuations and cycles of each parameter. Oxygen, total ammonia, CO_2, pH and temperature can all vary markedly over a 24-hour cycle (Wagner *et al.* 1995, Wurts 2003).
(2) The methodology of sampling. The method for taking the sample (e.g. to avoid aeration), the site from within the unit and any treatments (e.g. pre-filtering, chemical fixation) would need to be specified.
(3) The actual method for measurement. Most of the methods recommended (e.g. by the UK Standing Committee of Analysts: in the series of 'Blue Books'; and by the American Public Health Association) require a scientific capacity and equipment beyond the scope of most farmers. On-farm monitoring would therefore be dependent upon the availability of suitable probes and portable spectrophotometric kits. With appropriate guidance, fish farmers could reasonably be required to monitor temperature, DO and pH using probes; total ammonia nitrogen, alkalinity, nitrate and nitrite using field spectrophotometers; and CO_2 and un-ionised ammonia levels via computer packages after input of the required measurements. Farm measurement of gas supersaturation and suspended solids does not appear to be practicable. The water quality parameters that a farm manager could reasonably be expected to measure may reflect the level of intensity of the operation.
(4) Any additional calculation methods (if required).
(5) The unit for concentration (particularly important for nitrogenous compounds).

The second problem is that the setting of appropriate limits is inherently difficult. The numerous toxicological studies assessing the physiological tolerance of

farmed fish to various water quality parameters often give disparate or conflicting recommendations for safe levels. This is the result of complex interactions of water chemistry affecting the actions of fish, and the numerous endogenous factors that affect the response of fish. For example, common minimum recommendations for DO are 5–6 mg/L, yet no mention is made of temperature and the effect that has on the capacity of the water to retain DO. Similarly, recommendations for maximum concentrations of ammonia make no allowance for reduced DO concentrations, and safe levels of nitrite must consider the chloride concentration of the water. It appears that the inconsistencies in reported toxic levels of metabolites in fish are primarily due to differences in water quality between tests, as concluded by Meade (1985). Furthermore, acute toxicity tests follow guidelines that attempt to standardise results (Randall & Tsui 2002). Such highly controlled experimental studies will also be highly biosecure, so effects of water quality on disease susceptibility will not become apparent. While the need to standardise test results is understandable, the conditions under test bear little resemblance to those found in commercial aquaculture practices. The relevance of using the test results in guidelines for water quality recommendations to protect fish welfare on commercial farms must therefore be questioned.

The imposition of single all-encompassing water quality limits derived from highly controlled experimental results is therefore problematic, as it could not be considered to have a strong scientific basis. An additional consideration is that a safe limit will depend upon the duration of exposure. Hence it would be appropriate for tolerable levels during short-term events such as handling and transport to be different to those during routine rearing.

Animal-based parameters

Animal-based parameters represent the response of the animal to the environment and therefore have the potential to circumvent uncertainties in relation to appropriate parameter limits. Such 'welfare indicators' have great potential as they provide a direct assessment of how the animal is coping with the environment, avoid problems associated with water quality monitoring, and represent an integral part of good stockmanship. The animal-based parameters brought up in this chapter can be categorised into behavioural, morphological and production indicators (Table 10.4).

These indicators differ in their response time, sensitivity and specificity to a particular parameter. The behavioural and production indicators are non-specific responses, and may have other possible causes than poor water quality. The morphological indicators are more specific for water quality problems, although various gill abnormalities are non-specific responses (Fivelstad *et al.* 2003b). Although providing a clear signal that the fish have been exposed to poor water, they are only apparent after adverse exposure.

Upon initial water quality deterioration, it is the physiology of the animal that is likely to respond first. However, physiological measures have not typically been

Table 10.4 Animal-based indicators of poor water quality.

Category	Indicator	Water quality problem
Behaviour	Aggregation near surface or inlet	Low DO
	Increased ventilation rate	Low DO; High ammonia
	Gaping mouths, flared operculae	High CO_2; low DO
	Decreased food intake	High ammonia
	Hyper excitability	High ammonia
	Violent erratic swimming	High ammonia
	Loss of equilibrium	High ammonia
	Moribund, lethargy, unresponsiveness, disorientation	High CO_2; high nitrite
Morphology	Bright red gill lamellae	High CO_2
	Gill damage	High ammonia; high nitrite; high suspended solids
	Brown blood	High nitrite
	Thymus damage	High nitrite
	Developmental abnormalities	High nitrate, temperature
	Nephrocalcinosis	High CO_2
	Gas bubble disease	Supersaturation
Production	Decreased growth	Low DO High ammonia
	Increased food conversion ratio	Low DO High ammonia
	Mortality	Lethal levels of any parameter

examined in studies of water quality, and it has been suggested that: 'normal haematological status in fishes may represent a value range so broad as to be meaningless' (Houston 1990). Physiological measures are therefore of little value as predictors.

If poor water has sublethal effects on fish physiology, behaviour and morphology, then it is highly probable that these will manifest in a reduced growth rate and increased food conversion ratio, as documented for dissolved oxygen and ammonia. These are perhaps the most sensitive animal-based indicators of poor water quality. However, growth is highly dependent upon temperature and photoperiod (as well as *inter alia* fish size, strain, diet quality, life stage) and baseline 'normal values' have yet to be established for many fish species.

Diseases, both non-infectious and infectious, are very good indicators of environmental quality in relation to health. Some non-infectious diseases are specific to particular parameters, e.g. gas bubble disease (supersaturation), methaemoglobinaemia (nitrite), and nephrocalcinosis (CO_2 and hardness). Environmental gill disease is acknowledged to be due to poor water quality, but the contributory parameters are not well defined (Wedemeyer 1996). Fin erosion has been linked to various water

Table 10.5 Infectious diseases of fish and predisposing water quality parameters (from Wedemeyer 1996).

Disease	Predisposing water quality parameter
Bacterial gill disease *(Flavobacterium* spp.)	Low oxygen (< 4 mg/L), elevated ammonia (> 0.02 mg/L)
Furunculosis *(Aeromonas salmonicida)*	Low oxygen (< 5 mg/L)
Bacterial kidney disease (BKD) *(Renibacterium salmoninarum)*	Water hardness < 100 mg/L
Infectious pancreatic necrosis (IPN)	Water hardness

quality parameters, i.e. low dissolved oxygen and alkalinity, and high ammonia and suspended solids (Bosakowski & Wagner 1994, Wedemeyer 1996). The gills are recognised as a primary route of antigen uptake (Zapata *et al*. 1987, Moore *et al.* 1998). An increase in ventilation rate due to reduced water quality will increase the volume of water (and number of water-borne pathogens) passing through the opercular cavity and damage to the gill epithelium will increase the risk of uptake of pathogens. Poor water quality has been implicated in the development of a variety of facultative (e.g. *Saprolegnia*, Carballo *et al.* 1995) and obligate fungal, bacterial and viral diseases (Table 10.5).

Although effects of water quality on disease susceptibility are frequently cited, hard scientific evidence is lacking. A notable exception is an epidemiological survey of *Aeromonas* spp. infection in trout hatcheries in northeast Spain (Ortega *et al.* 1996). This study found an association between the prevalence of *Aeromonas* spp. infection and dissolved oxygen and ammonia levels. These water quality parameters were suggested to act as risk factors at respective concentrations of <7 and >0.05 mg/L. Such determination of risk of disease on farms provides a possible method for determining appropriate levels for water quality parameters.

Mortality rate can be used as an indicator of the nature of a problem (Wedemeyer 1996). Very high mortalities within a short period (e.g. >50% in <1 day) indicate oxygen depletion or acute toxicity; high mortalities over a longer period (e.g. 50% in 5 days) indicate a virulent disease; and low mortality over an extended period (10% in 7 days) indicates poor environmental conditions (Wedemeyer 1996). Mortality can therefore be used as an indicator of water quality problems.

Safeguarding trout welfare

If fish farmers identify a problem with water quality, then remedial management options should be available (Table 10.6). For sudden acute problems due to system or supply failures this may include back-up supplies for oxygenation, aeration, water pumping etc. Operating near maximum production capacity equates to operation at maximum risk, so monitoring and back-up systems should be related to intensity of production.

Table 10.6 Possible management options to alleviate water quality problems (after Masser *et al*. 1999).

Water quality problem	Remedial management option
Low dissolved oxygen	Increase aeration Increase oxygenation Increase water exchange Feed daily ration over longer period Stop feeding Reduce stocking density
High carbon dioxide	Increase aeration Increase water exchange Add stripping column
High ammonia	Increase water exchange Reduce feeding rate
High nitrite	Increase water exchange Reduce feeding rate Add chloride

There have been calls for the introduction of legislation to preserve fish welfare (FAWC 1996, Lymbery 2002). The apparent simplicity of setting prescriptive limits for environmental quality is tempered by the problems of arriving at appropriate limits and the ability of farmers to self-monitor as discussed above. Wedemeyer (1996) recognised the complexity of setting limits, but nevertheless set out recommendations for water quality limits as a guideline to fish culturists (Table 10.7). These limits were intended as a framework for the prevention of disease in aquaculture, and were based upon a combination of experimental toxicological studies and experience of farm environments. Although the latter basis may be scientifically questionable, it does represent a huge resource of information derived from the real world.

The introduction of legislation for within-unit water quality would represent another legislative imposition on farmers. However, it could not be considered too different from existing legislation regulating discharges. Currently, fish farm effluents are monitored for dissolved oxygen level, increases in biological oxygen demand, and levels of ammonia and suspended solids.

The parameters over which the farmer can exert a degree of control, and could potentially be expected to monitor are dissolved oxygen, carbon dioxide, ammonia and nitrite. Parameters that are largely outwith the control of the farmer include acidity, alkalinity, temperature, nitrate, hardness and heavy metals. These water quality parameters could therefore be considered during registration of a new fish farm, but would not need routine monitoring (unless required for calculation of other parameters). If water quality parameters were to be introduced, the experience of the UK's Environment Agency, and Scottish Environment Protection Agency (SEPA) in monitoring and enforcement would prove useful.

However, it must be stressed that these limits are guidelines, and rigid implementation would represent a highly precautionary approach. Also, such limits do

Table 10.7 Recommended water chemistry limits to protect the health of salmonid fishes (from Wedemeyer 1996).

Parameter	Recommended Limits
Acidity	pH6–9
Alkalinity	> 20 mg/L (as $CaCO_3$)
Aluminium	< 0.075 mg/L
Un-ionised ammonia	< 0.02 mg/L
Calcium	> 5 mg/L
Carbon Dioxide	< 5–10 mg/L
Chloride	> 4 mg/L
Chlorine	< 0.003 mg/L
Copper	< 0.006 mg/L (soft water)
	< 0.03 mg/L (hard water)
Dissolved Oxygen	6 mg/L (coldwater fish) 4 mg/L (warmwater fish)
Gas supersaturation	< 110% total gas pressure (< 103% salmonid eggs)
Nitrate	< 1 mg/L
Nitrite	< 0.1 mg/L
Total Dissolved Solids	< 200 mg/L
Total Suspended Solids	< 80 mg/L

not provide a guarantee of good fish health, as prediction is difficult due to complex interactions. This is well illustrated by a complex water quality problem that is emerging in Norwegian Atlantic salmon smolts (Fivelstad *et al.* 2003b). The increasing use of oxygenation has allowed more intensive rearing, with the result that carbon dioxide levels are higher. This has had a knock-on effect of lowering the pH (due to the poor buffering capacity of the low alkalinity water). The carbon dioxide and reduced pH are then thought to combine with low levels of aluminium to adversely affect the fish (reduction of growth rate and increased mortality, ventilation rate and incidence of gill lesions).

Due to the complex interactions between water quality parameters, it would be extremely difficult to introduce across the board limits. A possible solution to this would be to characterise farms on the basis of the water chemistry of the inflow water, and then set water quality guidelines based on the chemistry and the known interactions: for example, intensive farms with high stocking densities with hard, alkaline water would require strict ammonia regulations to protect the welfare of fish, while a similar farm with soft, acidic water would require stringent CO_2 regulations.

A possible scheme for practical monitoring of fish welfare would be for farmers to introduce their own routine diagnostic screening using animal-based parameters in addition to water quality monitoring, although monitoring such parameters would place an additional onus on the farmer. Routine morphological screening could be incorporated into a farm management plan, and be restricted to a macroscopic examination of the skin, gills and fins. Some animal-based parameters are already monitored by many farmers, for example growth, food conversion ratio and mortalities, however, the development of practical, on-farm welfare indicators is an area that requires further investigation. The gathering and management of such data could be considered part of best practice, and would allow greater traceability of the product and have quality assurance benefits for the farmer.

Conclusions

There is a lack of strong scientific data on appropriate levels for water quality parameters from commercial aquaculture situations. Water quality limits could be introduced for some parameters, but these would have to be ranges rather than single limits, and standardised protocols for measurement would need to be developed.

Farmers should be made aware of fish-based indicators of poor water quality, and should periodically conduct health screening. They should also be encouraged to record incidences of fish-based indicators and disease that relate to poor water quality, and use the experience to introduce and adapt farm-based management plans that apply to their local inflow systems and water.

Further on-farm research into the role of water quality in fish welfare is necessary.

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Chapter 11
Welfare of Fish During Transport

Peter J. Southgate

Introduction

The transport of live fish, often over extremely long distances for an extended length of time, is an inevitable feature of most areas of fish keeping. The shipping of stocks of tropical fish from the Far East to the aquaria of the West may involve a complex series of journeys by a variety of transport, while the journey of Atlantic salmon (*Salmo salar*) smolts from a land-based freshwater farm to a distant marine site may be equally protracted and convoluted. All aspects of this transport can have a detrimental effect on the welfare of the fish. From the initial handling and loading, through the conditions experienced during the journey to the eventual unloading, the fish may suffer physical damage, sub-optimal environmental conditions and stress. This chapter primarily considers the transport of farmed fish, with ornamental fish being dealt with specifically in Chapter 16, although the principles laid down here can be applied to all fish.

Transport systems

The principal systems for transporting live fish are road transporters, well boats and air freight including short haul helicopter flights.

Road transporters

Road transport of farmed fish is usually carried out in multiple purpose-built tanks on a road haulage vehicle. These tanks are usually constructed of fibreglass with a sealable loading hatch and a valve/pipe discharge point (Figure 11.1). Oxygen is supplied from on-board cylinders. Probes for continuous water quality monitoring – dissolved oxygen and temperature – are provided and usually monitored from within the cab of the vehicle. Smaller numbers of fish may be transported in single tanks and ornamental fish are frequently carried in sealed polythene bags in insulated boxes.

Figure 11.1 Typical road transporter for farmed fish.

Well boats

Well boats are becoming increasingly used in marine fish farming, both for transporting fish to on-growing sites and also for moving harvest-sized fish to central slaughter stations (Figure 11.2). Fish are pumped into chambers (wells) in the hull of the boat, oxygen is provided and water is circulated through these chambers, either as an open system with water being constantly replenished through open valves or, in some circumstances – due to biosecurity or the requirement to chill the fish – with closed valves giving 100% recirculation. A variety of water quality parameters are monitored and most well boats are equipped with continuous video monitoring of the fish.

Figure 11.2 Well boats are used for the transport of live fish to on-growing sites or slaughter stations.

Figure 11.3 Helicopters are used for short distance transfers of fish to on-growing sites.

Air freight

Transport of live fish by air is mostly confined to shipping ornamental fish across the world (see Chapter 16). Apart from the transport of ova and milt, very little carriage of food fish is by air. The exception to this is the movement of some fish by helicopter. This typically occurs when transporting Atlantic salmon smolts from land to on-growing sites at sea (Figure 11.3). Fish are loaded from road transporters into tanks slung beneath helicopters, and these tanks are then sealed and flown to the sea pens. The tank is then lowered into the water within the pen and a float mechanism on the tank releases the fish into the water. The journey time for this operation is relatively short.

Legislation and guidance

The principal legislation covering the transport of live animals in the UK, including fish, is the Welfare of Animals (Transport) Order 2006 (WATO), which came into force in January 2007. This puts in place procedures complying with Council Regulation (EC) N0 1/2005. WATO covers the transport of all vertebrate animals

when involved in any economic activity. Detailed provisions for fish are not set out but require that the animals are fit for transport and that the means of transport must be designed, constructed, maintained and operated so as to avoid injury and suffering and to ensure safety. Transport must provide protection from extreme temperatures and adverse changes in climatic conditions. If a container is carried loose in a vehicle this must indicate the right way to be carried, shaking is to be minimised and the container marked to show that it contains live animals.

Authorisations are required for transporting fish over 65 km. The transporter must have no record of serious breaches of animal welfare legislation. The transporter must have been suitably trained and be able to demonstrate that they have appropriate staff and equipment to transport animals. WATO principally applies to road vehicles and does not specifically relate to well boats or helicopter transport. It only applies to that which is of a commercial nature. It does not apply to an individual animal accompanied by a person who has responsibility for the animal and it does not apply to pets on a private journey.

The Farm Animal Welfare Council produced one of the first detailed reports into the welfare of farmed fish (FAWC 1996). The report detailed a number of specific recommendations for the transport of salmon and trout to the UK Government including the following:

- The fish should be regularly inspected
- Dissolved oxygen level should be maintained above 6 mg/L
- Carbon dioxide to be kept low and excessive changes in pH and temperature avoided
- Delays should be minimised
- Food deprivation prior to transport is recommended
- Crowding prior to transport should be minimised
- Only fit and healthy fish to be transported
- Appropriate records to be maintained

Subsequent to this report, other publications have included recommendations on the transport of farmed fish, for example, the Code of Good Practice For Scottish Finfish Aquaculture. These build on the FAWC report but avoid giving specific recommendations regarding, for example, stocking density and water quality parameters.

With the rapid increase in fish farming towards the end of the twentieth century, there was a parallel development of retailer and independent codes of practice detailing standards for the production of farmed salmon; some of these included requirements for transport. The Royal Society for the Prevention of Cruelty to Animals (RSPCA) has produced welfare standards for Freedom Food Salmon which has detailed requirements on transport, including the use of well boats for harvest, defining levels for water quality parameters, rate of chilling of the water in well boats and maximum stocking densities. A similar report for trout is due to follow. The Humane Slaughter Association has also published Guidance Notes for Humane Harvesting which includes welfare aspects of the use of well boats.

A great deal of the above is specifically aimed at farmed salmonids and, although general legislation and guidance can be applied, there are no specific references to other farmed fish species in the UK.

The impact of transport on fish welfare

If examined in the context of the five freedoms (FAWC 2005), transport has a potential impact on all five:

(1) Freedom from hunger and thirst – fish are deprived of food prior to transport and, if thirst is equated with osmoregulatory disturbance, fish lose salt during transport, which may have an effect on water balance (Wurts, 1999).
(2) Freedom to express normal behaviour – relatively high stocking density necessary for transport will allow less space for normal swimming behaviour.
(3) Freedom from fear and distress – handling and physical disturbance will inevitably occur.
(4) Freedom from pain, injury and disease – handling, physical disturbance, inadequate biosecurity and high stocking densities may lead to damage or a greater exposure or vulnerability to pathogens.
(5) Freedom from discomfort – there is potential for changes in water quality and environmental conditions, including vibration and physical disturbance, to cause discomfort.

The impact on welfare will vary according to a number of factors, many of which are interrelated as follows.

Fish factors

There is a legislative requirement that fish are fit for transport. Diseased or damaged fish must not be transported; this would include any which are considered to be unsuitable to transport, e.g. those with deformed jaws or gills which may be less able to tolerate transport conditions.

The ability of the fish to tolerate handling, crowding and physical disturbance varies with species, age and size and physiological condition. Atlantic salmon, for example, are far more susceptible to damage and stress at the time of smoltification and – by the very nature of the life cycle – this is when they are likely to be subjected to transportation.

Non-fish factors

The impact on the welfare of the transported fish will depend on the method of handling at the beginning and end of the journey, the quality of the water during transport, the stocking density, the duration of the journey, the weather (particularly in well boats), the degree of physical disturbance occurring during the journey and the degree of biosecurity present.

Human factors

Transportation is frequently in the hands of a third party and out of the control of the supplier or recipient of the fish. It is therefore important that personnel involved in transport are adequately trained in appropriate handling and environmental control during the journey; that they are aware of potential effects on fish welfare; that they are able to identify indications of poor welfare; and appreciate biosecurity risks. Research into the transport of terrestrial livestock has shown that the driving conditions in terms of acceleration, braking, cornering etc. have a direct effect on the stress and welfare of the animals (Cockram *et al*. 1997). It is highly probable that fish will be affected in the same manner, and is therefore important that transporters recognise this.

There may be particular concerns with transporting live fish to a central slaughter facility when there could be less concern for fish which are due to be killed on arrival. There is obviously the same need to minimise the impact on fish welfare in these circumstances as in all other fish transport, with the additional advantage of improved product quality if the fish are handled sympathetically.

There are various ways in which transport may have an impact on the fish being transported. These include stress, changes in water quality resulting in poorer environmental conditions, physiological changes, physical damage and disease.

Stress

All stages of transport, including handling during the loading stage, crowding and pumping/netting, protracted journey times often involving more than one vehicle and eventual unloading, are likely to cause a degree of stress. While a single episode of acute stress may not be inherently damaging, the additive effect of the series of stressors encountered during transportation may lead to the more chronic effect of immune suppression, increased susceptibility to disease and damage and a reduced healing response. The fish may therefore suffer adverse effects of stress during transport procedures which may predispose to physical damage and an increased risk of disease.

Research on loading and transport stress demonstrated that the stress response increased with transport time but that the act of loading and unloading was actually the more stressful part of the procedure (Svobodova *et al*. 1999, Carneiro *et al*. 2002, Kubilay & Ulukoy 2002).

Water quality

Transportation of fish is frequently carried out in 'static' water with very little chance of any water exchange. This applies principally to air and road transport. Well boats are able to exchange water during passage, although may need to recirculate water in the wells for biosecurity purposes during some parts of the journey. Where there

is no, or little, chance of water exchange during the journey, it is inevitable that certain water quality parameters will deteriorate. Waste products from the fish such as ammonia and carbon dioxide are likely to increase, as may the presence of organic material and suspended solids from faeces, and this will be exacerbated by the relatively high stocking densities involved. Oxygenation/agitation of the water and withdrawal of feed prior to transport will help to limit any deterioration in water quality but, particularly during long journeys, water quality is very likely to be compromised and this may have a detrimental effect on the health and welfare of the fish. Acute changes in temperature and pH shifts may also be experienced. Anecdotal evidence suggests that fish in helicopter buckets may have suffered acute temperature change which has affected their subsequent performance and survival at sea compared with similar fish transported by well boat.

Adequate oxygen must be provided and continuously monitored throughout the journey, but the provision of *too much* oxygen may also be detrimental to the fish, reducing their ability to exchange carbon dioxide (potentially leading to a metabolic acidosis) and also risking supersaturation problems.

All these changes in water quality can have a detrimental effect on fish welfare, as described in Chapter 10.

Physiological effects

During transport, fish continuously lose salts principally through their gills due to the effect of acute stress and loss of mucus protection (Wurts 1999). This may lead to osmoregulatory disturbance and possible tissue damage. Nothing is known of possible physiological effects of motion or altitude, such as may be experienced in the unpressurised hold of an aeroplane, but it is not inconceivable that animals with relatively sophisticated balance and buoyancy mechanisms may suffer motion or altitude ‘sickness’.

Physical damage

During transport, fish are at risk from suffering physical damage through handling, increased stocking density and the motion of the vehicle leading to descaling, fin erosion, snout abrasion and eye damage. Well boats subjected to severe sea conditions can result in extreme physical damage. Any such damage will increase the risk of osmoregulatory disturbance, render the fish more susceptible to disease organisms and compromise subsequent performance.

Disease

Transport equipment is used repeatedly to move fish between different geographic areas. The risk to biosecurity can be great unless very strict sanitation and biosecurity arrangements are maintained. The risk of spreading infectious disease and

precipitating disease outbreaks is exacerbated by the fact that stocks at risk may already be compromised by handling/transport stress, sub-optimal water quality, higher than usual stocking densities and by any damage they have suffered. It is therefore imperative that appropriate biosecurity measures are taken to minimise the risk of transmitting disease and that pre-transport health checks are carried out to ensure that only healthy individuals are transported.

Minimising the impact of transport on welfare

It is clear that transportation can have various impacts on the welfare of fish and that many of these factors are interrelated. Although, as indicated above, guidance is available for general procedures during handling and transport there is a need for further work and more detailed guidelines for optimum transport conditions, particularly for species other than salmonids. These guidelines should include recommendations for maximum duration of transport, water quality conditions, stocking densities and stress reduction. The possibility of equipment and vehicle breakdown must be recognised and ‘safety factors’ must be included in all transport procedures ensuring that welfare of the fish is not compromised by any delay or equipment failure. Training of handling and transport operatives is a requirement of legislation and the various codes of practice and it is vital that personnel involved in fish transport are sympathetic to the welfare of the animals under their care and are able to recognise indicators of poor welfare.

Various water additives have been proposed to reduce stress and damage during transport, these range from aloe vera and various plant extracts to sedation using anaesthetics (anecdotal reports, Berka 1986). The success of the use of these is debatable and, although sedation may ease handling during loading, great care must be exercised if using anaesthetics, as they may increase the risk of physical damage if the fish are unable to maintain their position in the water and withstand water movement within the transport vessel.

The use of salt in transport water has been more thoroughly investigated (Wurts 1999) and there is evidence that this will reduce the loss of salts from the fish during transport, may reduce stress and will also have some antibacterial, antifungal and antiparasite activity. Barton & Peter (1982) claim, however, that adding salt during transport has no effect on reducing plasma cortisol levels and question whether there is any reduction in stress.

The salt concentration of fish blood is relatively constant at approximately 0.9% and the addition of sodium chloride up to this concentration in transport water will reduce the osmotic stress on the fish and consequently reduce loss of salts from the blood. It is important that the salt concentration does not go above this level, and it is sensible to err on the side of caution with a maximum concentration of 8 g/L (0.8%). The use of sodium bicarbonate (to a maximum level of 200 mg/L) and other buffers have also been advocated to counteract pH shifts due to accumulated

carbon dioxide (Berka 1986, Johnson 2004). The tolerance to salt varies with fish species: salmonids, catfish and tilapia tolerate a wide salinity range, but care must be taken with other species and, if use of salts is intended, trials should be carried out to ensure the fish will tolerate prolonged exposure.

Monitoring of fish welfare during transport

To ensure that the detrimental effect of transport is minimised, continuous monitoring of the fish must be carried out as far as is practically possible. It is difficult to observe fish when they are contained in truck transport tanks and helicopter buckets, but fish in well boats are monitored by video and it should be possible to adapt this technology for road and air transport except, perhaps, in cases where ornamental fish are held in sealed, insulated containers. Continuous remote monitoring of water quality is essential for all transport vessels and measuring instruments must be accurate and calibrated before each journey. Personnel must be able to recognise early signs of stress.

Monitoring should start well before loading to ensure that the fish are healthy and fit for the journey, and should continue after delivery identifying any damage or poor condition, or losses due to any aspect of transport, loading or unloading. There should be adequate feedback to the supplier and transport company to ensure any problems or, indeed lack of problems, are recognised.

Conclusions

What is clear is that fish are subject to negative influences associated with transport, and we need to apply good practice to avoid as many of these as possible, even if it means extrapolating knowledge from terrestrial animals pending the results from specific research in fish. Such good practice must then be incorporated into industry codes of practice as is currently the case.

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Chapter 12

Disease and Medicines – the Welfare Implications

Tony Wall

Introduction

One of the consequences of the presence of disease in fish is often an increased level of mortality within a population. As well as the implications for these dying fish, it is usually accompanied by the presence of moribund fish showing clinical signs of the disease. So, while death itself is not a welfare issue, the process of dying for the diseased animal certainly is. Therefore, one of the most important factors to ensure good welfare standards is the ability to control disease. Although medicine use is only an adjunct to good husbandry, there will be times when it is necessary to control or eradicate disease with medication.

Disease: the welfare consequences

Although the process of dying is clearly a welfare issue for the diseased animal, there are also other factors associated with disease which need to be taken into account.

Presence of moribund and dying fish

In populations of farmed fish, moribund and dying fish should, if possible, be removed and humanely killed. While this is achievable for fish on or near the surface, removing affected fish or monitoring them deep within the water column is less straightforward. This action is important not only to alleviate any suffering in the affected fish but, in cases where a pathogenic agent is involved, also to reduce the numbers of infectious organisms being discharged into the water and so decrease the level of challenge to other fish in the population.

Decrease in water quality

Dead fish that are present in the tank or pen, besides acting as a source of infection will adversely affect the quality of the water due to the release of breakdown

products from putrefaction and decay. This in turn will compromise the live fish making them more susceptible to those diseases already present.

Demand on human resources

Mortalities should be removed at least daily, often putting a great strain on the resources of the farm staff. If jobs such as feeding, grading and net changing are hurried or delayed as a result of the extra time taken in collecting and removing dead fish, then the health and welfare of the live fish might suffer. With increased automation in most aquaculture systems, and the consequent reduction in staff numbers, one of the most important jobs, that of observing and monitoring fish behaviour and signs of disease, is not given the priority it deserves.

Potential to spread to wild fish

Disease in farmed fish caused by pathogens (virus, bacteria, parasites) has the potential to spread to other species and populations. For example, sea lice from farmed salmon have been said to infest wild sea trout and salmon. While we usually have the resources to treat the farmed fish under our care, we are not in a position to treat wild fish which might be affected by farming practices. This is an ethical dimension that we must recognise.

Effects of food withdrawal

Some fish diseases can be ameliorated by the withdrawal of food for a few days. The decrease in metabolic by-products in the water as well as the lowered metabolic rate of the fish themselves can slow down the rate of development of disease. However, starving fish for a few days, which have been used to being fed regularly, has the potential to affect their welfare. Usually this small cost is easily outweighed by the reduction in disease and suffering.

Medicines: the welfare implications

Although medicines, used appropriately, can be very effective at controlling or eradicating disease, and therefore can be good for welfare, there are several aspects to their use which must also be considered in this context.

Lack of fish medicines

Over the last few years there has been a significant reduction in the numbers of medicines available for use in aquaculture. The antimicrobials furazolidone, oxolinic acid and sarafloxacin have been withdrawn leaving only four antimicrobials

for aquaculture use in the UK: amoxycillin; potentiated sulphonamides; oxytetracycline; and florfenicol. Similarly, the sea lice products dichlorvos and hydrogen peroxide are no longer licensed and, at the time of writing, azamethiphos and teflubenzuron are not available, leaving only cypermethrin and emamectin benzoate. This low number of available medicines leads to greater reliance on the few which are available, with the consequent risk of a build up of resistance. This in turn leads to increased frequency of use as the medicine becomes less and less effective, with a consequent impact on welfare.

The reasons for the low numbers of medicines available are many and complicated. The cost of maintaining a licence, consumer safety issues and environmental considerations are some of the entirely valid reasons for loss of fish medicines. However, safe and effective medicines must be available for the viability of the industry and not least, the welfare of the fish.

Environmental constraints

Fish medicines, in contrast to medicines for terrestrial animals, require an environmental assessment from the relevant authorities before they can be discharged into water. Although an environmental assessment will have been carried out by the marketing authorisation holder and evaluated by the Veterinary Medicines Directorate (VMD), the environmental authorities also look at the discharge of all aquaculture medicines on a site-by-site basis before they can be used. This can lead to the frustrating scenario for the farmer and vet where a licensed medicine cannot be used on a particular site due to the lack of a consent to discharge.

Possible side effects

All medicines have the potential to cause side effects or adverse reactions and fish medicines are no exception. For example, the development of adhesions following intraperitoneal vaccination and the risks associated with bath treatments for ectoparasites are well understood. Bath treatments can cause both physical damage and stress. Consequently, use of medicines themselves can have negative welfare implications, but if we balance the risks of the treatment over the benefit in disease control, the cost to welfare associated with these treatments is small compared to the devastating effect diseases can have.

Withdrawal periods

The depletion of medicines from the fish is necessary before a fish can be presented for human consumption. The withdrawal periods (usually in degree-days) set to guard against the possibility of residues remaining, can lead to a situation where a population of fish require medication but are too near to harvest to treat. The farmer

then has the option of treating and delaying harvest, or bringing the harvest timing forward. The welfare of the fish would be compromised if the disease was allowed to progress untreated through the population in order to allow the harvest to take place at the scheduled time.

Retailer and organic constraints

One of the unexpected constraints in the proper use of medicines in aquaculture can be from the retailers or organic associations who limit the use of some licensed medicines in fish farming. These are licensed medicines with full marketing authorisation that have been carefully assessed for quality, safety and efficacy. Forbidding the necessary use of medicines or arbitrarily increasing the length of the withdrawal period may have the perception of benefiting the consumer, but does little to improve the welfare of the fish.

Delay in treatments

Until recently, the on-farm mixing of medication was a speedy method of ensuring the fish were treated as soon as possible in the face of a sudden outbreak of disease. Recent legislation has curtailed this on-farm mixing to some extent and most farms are now relying on the feed manufacturers to mix the medication with the food. This can lead to inevitable delays with a consequent impact on the welfare of the fish being treated.

Treatment costs can be a factor in this problem as well. The high costs of medicines, and the costs associated with arranging and carrying out treatments, might lead to delays in the hope that the treatment may turn out to be unnecessary.

Disease and treatments: examples

Sea lice

Sea lice are arguably one of the most important welfare (and economic) problems that salmon farmers have had to deal with (Plate 4). With the advent of new efficacious medicines, it is most uncommon to see fish severely damaged by sea lice. These medicines, which are in-feed and bath treatments, when compared with previous treatments are safer for the fish, the operator and the environment, and have resulted in levels of ovigerous lice often being less than one per fish. This has had a significant positive welfare benefit for the Atlantic salmon.

At the moment, however, there are only two sea lice treatments available, and overuse of these products is selecting for drug resistance. In addition, due to the environmental constraints, the quantity that can be discharged at any one time for bath treatments can be less than is needed to treat the whole site. This can result in

untreated fish being exposed to a sub-therapeutic dose from the neighbouring pens which are treated, leading to increasing tolerance to the medicine. Additionally the lice from untreated fish can resettle on the post-treated fish.

The entirely justifiable environmental concerns over the use of the medicines can be in conflict with those actions which will best serve the welfare of the fish. Consequently it is important that good dialogue is maintained between interested parties, to ensure best practice is established.

Furunculosis and vaccination

Fifteen to twenty years ago, the welfare and economic implications of the bacterial disease furunculosis were severe. Large numbers of salmon, often up to 20–30% of a population, might be affected and die. Antimicrobial therapy controlled (just) these outbreaks, although with economic and environmental costs. It is significant that since the development of furunculosis vaccines, the amount of antimicrobials used has decreased to less than 5% of the levels 20 years ago (VMD 1990–2005), with an associated major improvement in fish welfare.

However, these same vaccines are given by intraperitoneal injection, and can produce a reaction at the injection site causing adhesions, melanisation and scarring (Plate 5). The slight decrease in performance of the vaccinated fish, as well as the temporary loss of appetite would suggest that there is some adverse affect on their welfare.

On balance, the furunculosis vaccination has undoubtedly been beneficial for fish welfare but these adverse effects must be a driver to investigate new products with fewer side effects.

Production diseases

This group can loosely be defined as diseases caused or exacerbated by the production methods themselves. Improved technology, especially during egg incubation, first feeding, and in water recirculation systems, have resulted in accelerated growth in the early stages of the fish's life. Although the aetiology is probably multifactorial in origin, there is some evidence to suggest that this improved growth rate, (or the environmental conditions that have stimulated the increased growth), has been responsible for some of the deformities seen in farmed fish (see Chapter 13). These conditions often appear as occasional sporadic episodes, but can also occur at constant 'background' levels. Spinal deformities, cataracts and jaw malformations (Plates 6 to 9) could thus be classified as production diseases. Inability to swim, to feed efficiently or to have good vision may not necessarily affect the performance of the fish, but such defects are ethically questionable and contribute to poor welfare.

While there are few, if any, treatments available, production disease can often be controlled by husbandry changes. However, this might require a scale back in production with economic consequences.

Ectoparasitic diseases

Skin and gill parasites are common in most aquaculture systems, particularly in fresh water, leading to skin irritation, self-mutilation, loss of performance and death. At summer temperatures, there is the potential for epizootic disease, especially from infestations upstream of the farm.

In the UK at the moment, with the exception of sea lice, there are no licensed medicines to treat these parasites in sea or fresh water. Husbandry expertise as well as traditional remedies such as formalin have, in some cases, controlled outbreaks but a number of farms have suffered huge losses. These diseases can and do have a serious impact on fish welfare, and there is a real and urgent need for licensed medicines to treat these conditions.

Water quality

In intensive recirculation systems, the fish farmer has near total control over the quality of the water. Oxygen, ammonia, carbon dioxide, total gas saturation, suspended solids and nitrites will be monitored and controlled. In more extensive systems using a one pass water supply, as well as cage systems, it can be difficult to maintain good water quality.

Any deterioration in water quality will have some effect on the fish, and so will have an impact on fish welfare. For example, slightly raised levels of carbon dioxide may only manifest as a loss of performance, whereas sudden supersaturation of the water can lead to serious losses as well as chronically affecting some fish (Plate 9). Due to the uncontrolled ingress of water, fish farmed in pens are particularly at the mercy of the quality of the surrounding water. Jellyfish in the sea and algal blooms in sea or fresh water, can be difficult to control and can cause oxygen depletion and/or toxin production.

Conclusions

The presence of disease in farmed fish populations has severe welfare implications for affected fish, and poses a threat to the welfare of unaffected fish. Resources to reduce this threat by the removal of dead and dying fish are often not available due to financial/management constraints, thus causing an ethical dilemma. Diseases can potentially be treated on farms, but wild fish around the farms which may become infected cannot be treated, so an ethical dilemma also exists over any impact in the wild stock.

Treatment of a disease may be achieved by food withdrawal, which itself has an impact on welfare, but this is normally outweighed by the benefits achieved. Similarly, where medicine use results in side effects which have welfare implications,

these negative effects are normally outweighed by the positive effects of the treatment.

Although medicines are available for the treatment of some diseases, they are few in number, and this can result in disease resistance development and increased need for treatments, with a consequent impact on welfare. There are many reasons for this paucity of supply, but even when medicines are available, they sometimes cannot be used for want of a discharge consent, or their use may need to be delayed due to restrictions on medication of feed, or avoided due to constraints related to withdrawal periods.

So the farming of fish when disease occurs will inevitably affect the welfare of the fish, and medicines will not always be available to treat the diseases. When they are available, there may well be constraints on their use. Consequently, more safe, effective medicines are necessary, along with improvements in husbandry and management which will reduce the need for those medicines.

Reference

VMD (1990–2005) *Sales of Antimicrobial Products Authorised for Use as Veterinary Medicines, Antiprotozoals, Antifungals, Growth Promoters and Coccidiostats in the UK*. Veterinary Medicines Directorate, London.

Chapter 13
Welfare and Deformities in Fish

Edward J. Branson and Tom Turnbull

Introduction

Deformities have been described in many species of fish worldwide, in both farmed and wild stocks, for a long time (e.g. Dawson 1964, 1966, 1971, Dawson & Heal 1976) and, although often spoken of in relation to aquaculture, it is important to note that the presence of deformities is by no means a farmed fish phenomenon, or an unusual occurrence. This may well be related to the fact that almost all fish eggs undergo development in an unprotected environment, so the process of embryogenesis occurs subject to the many and varied influences that the environment might introduce. There are probably almost as many theories as to the cause of deformities as there are deformities, and almost any factor imaginable has come in to the list of possible causes at some time or other, but only a few have been proven.

Deformities can have a serious impact, not only because of the obvious welfare and ethical implications for affected individuals but, related to aquaculture, there may also be considerable costs involved for producers. Consequently, quite intensive research has followed in response to some problems.

Although this chapter will consider deformities in the context of farmed fish, particularly farmed salmonids, the type of deformities described are likely to be seen in a whole variety of farmed and wild fish. There are too many to cover adequately here in detail, particularly the very minor problems, so a few examples of particular interest because of their obvious appearance or financial importance will be considered.

Soft tissue deformities

A number of soft tissue deformities have been recorded in salmonids, particularly related to the heart, septum transversum and abdominal organs.

Heart abnormalities

Heart morphology

Farmed salmonids often have more rounded hearts than wild fish (Plate 10). Poppe *et al*. (2003) found that the heart shape, when compared to wild fish, is more variable

in farmed salmonids, the ventricle being more rounded and the bulbus arteriosus misaligned. Also, the angle between the ventricular axis and the bulbus arteriosus is generally more acute in wild fish than farmed.

The shape of the heart varies between different types of fish, largely reflecting the behaviour and activity of the species, and the morphology of the heart has been shown to correlate closely with its required function. In common with other highly active or migratory species, the normal salmonid heart has a large, sharply triangular ventricle with a well-developed outer compact myocardium, designed to pump with quite a small stroke volume under high pressure at a relatively high heart rate. A strong positive correlation has been shown to occur between this shape and optimum cardiac output (e.g. Graham & Farrell 1992, Tota & Gathuso 1996). Consequently, the result of an abnormal heart shape is probably that the pump efficiency is reduced so that misshapen hearts may not be able to meet sudden increases in demand. Unpublished reports from Norwegian veterinarians suggest that otherwise healthy fish with misshapen hearts are less able to cope with routine handling when compared to fish with normal shaped hearts, and suffer from unacceptably high mortalities (Poppe *et al.* 2003). Similar findings have been reported in Canada (Brocklebank & Raverty 2002). Therefore, this condition may not adversely affect the welfare of affected salmon in stable conditions, although poor growth rates may be seen (Poppe *et al.* 2003), but an effect will almost certainly be seen in stressful situations.

The cause of this abnormality is unknown, but Poppe *et al*. (2003) suggest that the rounded heart shape could be a result of breeding, fast growth, a sedentary lifestyle, or a combination of all these factors. Certainly lifestyle can affect cardiac morphology, for example Graham & Farrell (1992) showed that the thickness of the compact myocardium in rainbow trout varies with environment and ‘lifestyle’, and Powell *et al*. (2002) showed that the ventricular compact myocardium of Atlantic salmon (*Salmo salar*) became thicker with heavy infestations of amoebic gill disease.

Poppe *et al*. (2003) considered the misalignment of the bulbus arteriosus to be a probable direct consequence of the altered heart shape, and a further factor in the restriction of cardiac output during periods of increased demand.

Abnormal heart position

Heart position in Atlantic salmon can also be abnormal, with the apex lying dorsally in the pericardial cavity (Plate 11), so-called *situs inversus* of the heart. In this case the ventricle may be rounded or laterally compressed and bean shaped, or tubular, with stretching of the afferent and efferent vessels. This condition has also been reported in rainbow trout (*Oncorhychus mykiss*) broodstock (Poppe *et al*. 2002).

It is difficult to determine how significant this type of abnormality is for the fish, and it is certainly not always incompatible with normal life, but affected fish seem to tolerate stress less well than unaffected fish. This is probably due to the abnormal heart shape rather than its position because, as noted above, there is a strong

positive correlation between the normal salmonid heart shape and optimum cardiac output and function. Consequently the results of this abnormality are probably that pump efficiency is reduced, making sudden increases in demand difficult to satisfy. Stretching of blood vessels and their abnormal angles in relation to the heart may well exacerbate this problem. It seems likely that, as above, there will be few, if any, adverse effects due to this condition until stressful situations occur.

Absence of septum transversum

The septum transversum is the membrane separating the pericardial and abdominal cavities, and defects in this structure can occur, from partial to total absence (Plate 12). Norwegian health workers have reported a high prevalence of missing or incomplete septa in some farmed Atlantic salmon smolt groups, particularly in the 1996 year class (Poppe *et al*. 1998). Similar septal defects were also reported in Scottish farmed salmon over the same period (T. Turnbull pers. com.). Affected fish were certainly disadvantaged, and generally grew more slowly and with higher losses than unaffected siblings. In these fish the ventricle was commonly misshapen, being either pear or bean shaped, often misplaced, and sometimes adherent to the liver (Plate 12b). In some fish the ventricle may also be adherent to the abdominal wall due to diffuse post-vaccination peritonitis (Poppe *et al*. 1998).

Again, as noted above, the abnormal heart shape in this condition is likely to reduce cardiac efficiency and output in times of increased demand. This problem would almost certainly be exacerbated in cases where there was also adhesion of the heart to the liver or abdominal wall. In addition, the absence of the septum will have an additional impact on cardiac output because full cardiac function is dependent on the development of reduced pressure in the pericardial cavity during ventricular systole (Farrell & Jones 1992, Agnisola & Tota 1994), and this is unlikely to be fully achieved with this deformity. Consequently, the lack of a complete septum transversum is likely to have an impact on affected fish in times of stress, and the reduced pumping efficiency would probably account for the failure to thrive and, as above, would almost certainly reduce their ability to cope with stressful situations.

There may be many causes of this problem, but egg incubation temperature has been shown to be at least one of them (see below).

Other heart abnormalities

Other cardiac abnormalities have also been described in farmed salmonids, although not to the same degree as those mentioned above. For example, ventricular hypoplasia was described in Norwegian Atlantic salmon parr and pre-smolts in 1997 (Poppe & Taksdal 2000). In this condition, the ventricle was significantly smaller in relation to the atrium when compared to normal fish, with poor development or absence of the outer, compact myocardium.

Lesions of the coronary artery have been described in wild as well as farmed salmonids (e.g. Saunders *et al*. 1992, Davie & Thorarensen 1996) and, although

this is not apparently associated directly with farming, it does seem to be related to speed of growth (Saunders *et al*. 1992), so the fast growth seen in farmed salmon may predispose to the condition. The cause of these abnormalities has not been identified.

Other internal deformities

Swim bladder abnormalities

Swim bladder abnormalities may occur, for example with torsion or retroflexion where the swim bladder may be seen coiling and folding back on itself, or it can be displaced with atresia, or simply truncated (Plate 13). In Atlantic salmon, many affected fish seem to be able to lead completely normal lives with no externally abnormal signs (Plate 13a). In others, a problem may only become obvious when there is over-inflation of the swim bladder, which may be stress induced (as shown in Plate 13b and c at smolt transfer). In the particular case shown, and in others like it, mortalities only occurred when the swim bladder became over-inflated, but they were few in number. In other cases the deformity may lead to behavioural abnormalities, with affected fish often otherwise normal. For example the rainbow trout shown in Plate 13d had swim bladder abnormalities and was a 'side swimmer', that is, it normally swam on its side. However, side swimming is not always associated with swim bladder abnormalities, so the finding may have been incidental.

This problem is not confined to salmonids. For example, it has been seen in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). Chatain (1994) reported a lack of functional swim bladders in larval sea bass and bream, possibly related to an inability of the larval fish to fill their swim bladders in intensive rearing conditions. This swim bladder problem was thought to then lead to lordosis in affected fish. There was also an increase in mortality, which may have been related to a decrease in predatory efficiency and an increase in energetic needs.

Again, this condition is not necessarily incompatible with normal life, as the harvest fish in Plate 13a shows, although where losses occur there is a clear impact on welfare. Also, where affected fish cannot behave normally, it is possible that there may be some implications for the psychological well-being of the animals.

Causes of this problem are unclear, but egg incubation temperature has been shown to have some impact on incidence (see below).

Right-handed liver/Situs inversus of the liver

The liver can sometimes be misplaced, lying dorsally above the stomach, or on the right side of the anterior abdominal cavity rather than the left, as in the normal fish (Plate 14). This can occur alone, or the position of all the internal organs may be reversed. Affected fish usually show no adverse effects of simple liver transposition, but apparent poor thrift may possibly occur. However this could be accounted for by the presence of multiple defects which are sometimes seen in the same fish, for example the liver displacement may be seen with septal defects and/or a reduced number of pyloric caeca (T. Turnbull pers. com.).

Again, the causes of this problem are unknown, but egg incubation temperature has been shown to be at least one of them (see below).

Aetiology of soft tissue deformities

The incidence of most of the deformities mentioned above can be strongly influenced by the temperature at which eggs are incubated in salmon (Baeverfjord *et al*. 1998a) and rainbow trout (Baeverfjord 2003). Egg incubation temperature was found to influence the prevalence of: defects in the septum transversum; inverted hearts; swim bladder torsion; abdominal organ *situs inversus*; and reduction of pyloric caeca in number and size. It was also shown to have an impact on the incidence of some skeletal abnormalities. The recommendations for incubation temperature which have arisen from this work are to keep incubation temperatures up till first feeding to below 8°C for Atlantic salmon and 10°C for rainbow trout. These recommendations have been widely adopted by the salmon industry and, as a consequence, some of these deformities are now rare.

Other work has suggested that high levels of vitamin A may have a modifying influence on the incidence of soft tissue abnormalities in salmonids induced by high temperatures, such as missing septum transversum and *situs inversus* of the heart, but this was not conclusive (Ørnsrud *et al*. 2004).

Abnormalities induced by inappropriate incubation temperatures have also occurred in other farmed species. For example, Bolla & Holmefjord (1988) reported the incidence of mouth deformities in halibut exposed to high incubation temperatures, with more affected in a group kept at 10°C than in groups kept at 6°C and 2°C. In this case, the presence of light during incubation also increased the incidence of deformities. Wiegand *et al*. (1989) reported a decreased survival to hatching and an increased frequency of abnormal development when goldfish embryos and larvae were exposed to incubation temperatures of 13°C, either constantly or with daily fluctuations between 22°C and 13°C, when compared to a normal incubation temperature of 22°C.

Clearly, some of the factors influencing the incidence of these soft tissue deformities have been determined, but others remain unexplained, e.g. for misshapen hearts, ventricular hypoplasia and coronary arteriosclerosis in salmonids. Consequently there are obviously other factors, as yet unknown, in what seems to be a problem with a complex aetiology.

Skeletal system

A number of deformities of the skeletal system have been recorded in fish, particularly in salmonids, involving the gill covers, jaws and, of particular importance, the spine.

Gill cover defects (shortened opercula)

Gill cover defects consisting of curling or folding and abnormal formation of the opercula are often described (Plate 15) and can be uni- or bilateral. The condition can sometimes be associated with erosion of the opercular edges, but this is by no means always the case. The result of this abnormality is that the fish cannot seal the gill cavity to create the suction necessary for operation of the normal buccal pump ventilation system. The fish are also unable to 'cough' if necessary, to clear debris from the gills. In addition, the gills may be exposed and therefore could be susceptible to damage, possibly resulting in the shortening and thickening or deformation of gill filaments often seen in fish with short opercula.

The inability to operate the buccal pump means that fish may have to swim continuously in order to maintain an adequate flow of water over the gills. This makes affected fish particularly susceptible to handling which involves netting or any other form of containment. Consequently, this condition tends to make affected fish less tolerant of handling or poor conditions, and the fish's tolerance to stress is reduced.

The condition is not uncommon in farmed salmonids, although numbers affected are usually low. Other species can also be affected, for example, the opercular complex deformity (OCD) can be a real problem in juvenile sea bream (Koumoundouros *et al*. 1997). This condition is first seen in larvae, and develops fully during metamorphosis, with severe folding and twisting of the operculum. A non-heritable lack of the posterior edge of the operculum has also been reported in Mozambique tilapia (*Tilapia mossambica*, Handwerker & Tave 1994).

A definitive cause of the shortened operculum problem has not been determined, but damage to the free edges of the opercula due to, for example, trauma associated with netting, can lead to erosion of the opercula edges with consequent shortening. In addition, egg incubation temperature has been shown to have a significant influence on the occurrence in salmonids (Baeverfjord *et al*. 1997). A high prevalence in some populations of Atlantic salmon derived from imported Tasmanian eggs has been seen in Scotland (T. Turnbull pers. com.), and this stock type bias suggests a possible heritable element to the problem. However, poor temperature control during 'out of season' incubation may have been more significant than stock type in this case.

Jaw deformities

Screamer disease

There was a high prevalence of lower jaw deformity in Atlantic salmon in Scotland in the winter of 1987–88 (Bruno 1990) and 1996 (T. Turnbull unpublished data) (Plate 16), and in Chile in 1998 and 1999: the so-called screamer disease (Roberts *et al*. 2001). Although a significant problem at times, occasional fish with this problem may also be seen in many populations in most year classes.

In this condition, the lower jaw and its articulation become deformed and jaw movement is restricted, often resulting in a fixed gape. Affected fish are unable to fully open or close their jaws, so can't feed properly and fail to thrive. In addition, in common with fish suffering from simple opercular abnormalities, these fish are also unable to operate the buccal pump mechanism making proper ventilation impossible, with inevitable mortalities during handling. This is made worse because, in this condition, gill covers are usually also very soft due to rarefaction of osseous and cartilaginous tissues, and can be deformed and sometimes shortened. Gill arch and spinal deformities were also reported from the affected populations.

More recently, a first gill arch deformity with a similar appearance to that seen in screamer disease has been reported in seawater Atlantic salmon in Chile, but this time with no other abnormalities associated with it (G. Ritchie pers. com.).

The investigation into the Chilean screamer disease (Roberts *et al.* 2001) indicated that the cause may well have been a marginal phosphorous and vitamin C deficiency in the feed coinciding with very rapid growth and high water temperatures. Phosphorous deficiency in fish has been shown to result in the presence of crooked and soft bones (Baeverfjord *et al.* 1998b), and this same jaw pathology has been reproduced experimentally in the laboratory (G. Baeverfjord pers. com.), along with vertebral abnormalities, by using sub-optimal mineral nutrition in early juveniles undergoing rapid growth. High temperatures were not necessary for the successful reproduction of the condition. These experimental results fit well with the findings in Chile, but are not so supportive of mineral deficiency as a cause in the cases of this problem in Scotland mentioned above. The significantly lower sea temperatures post-transfer, when the problem became apparent, and consequent poor growth rates contrast with the Chilean case (T. Turnbull pers. com.). However, growth rates in fresh water prior to transfer are likely to have been high, and early indications of jaw deformity could have been overlooked in fresh water, as they were in many cases in Chile. It was concluded that, although the ultimate cause remained unknown, the deformity was not related to any particular strain or origin of fish.

Consequently it seems likely that a multifactorial aetiology was involved with the outbreaks of increased prevalence of jaw deformity in farmed salmon, as indeed is likely to be the case with individual affected fish seen in most populations.

Pug nose (brachygnathia)

In this condition there is under-development of the maxilla, and a consequent projection of the mandible, giving the fish a prognathous appearance (Plate 17). Again, occasional fish affected with this condition will be seen in most populations, which is usually the extent of the problem. However, for affected fish, welfare can suffer if the condition is severe, as it can interfere with uptake of feed, but this is probably not a very significant issue for farmed fish where food is abundant.

A definitive cause of this problem has not been determined but, again, egg incubation temperature has been shown to have an influence on its prevalence (Baeverfjord *et al*. 1998a).

Spinal deformities

Spinal deformities of several different types can occur, and all can result in a visually altered shape in their extreme forms. Descriptions of spinal problems often refer to the change of shape without necessarily shedding any light on the cause(s) of these deformities, e.g. kyphosis, lordosis and scoliosis indicate an angular deviation along the length of the spine. In recent years, spinal deformities in farmed Atlantic salmon, variably described as short tails, humpbacks and stumpies, have become a significant production issue for some companies in Scotland and Norway, with a considerable financial impact. These problems appear to result from alterations in growth in the vertebral and intervertebral tissues, sometimes with intervertebral fusions – if those in the thoracic region are predominantly affected, the result is humpback (Plate 6), whereas the main effect in the tail region results in short tail, although both can occur in the same individual resulting in a stumpy.

Severely affected fish are poorer swimmers than normal fish, less agile, and less efficient at competing for food, so tend to be below average weight as populations approach harvest size. Although a reduction in flexibility of the spine is often apparent when affected fish are watched, whether these fish suffer pain or discomfort as a result of the pathological process is open to question. However, affected individuals do appear to have a lower tolerance to handling and other stressful situations, and the production of significant numbers of fish with these deformities raises ethical questions.

In addition to the ethical questions, the problem can also be very costly in financial terms. Harvested salmon with obvious spinal deformities are likely to be downgraded from 'superior' to 'ordinary', or even to 'production' grade if severe enough. These downgrades will normally be less valuable than 'superior' grade fish, and can thus have a significant impact on the profitability of salmon farms which rely on around 95% of harvest fish achieving 'superior' status.

Other financial costs are also associated with this type of problem. Harvesting and processing will both be slower. Machine efficiency is reduced, as equipment is designed for 'normal' shaped fish, so processing damage is increased and more manual processing and extra trimming is necessary with a resultant loss of yield. The appearance of the final product can also suffer with a consequent loss of value (Plate 18).

Spinal deformities have probably always been present within salmonid populations, and have certainly been recorded in wild fish for many years (e.g. Howes 1894, Gemmill 1912, Gill & Fisk 1966, Hansen & Yalew 1988). Consequently there will probably always be a background level in farmed fish. However, some populations of farmed fish have been particularly badly affected.

Spinal deformities were being recognised as a problem in some groups of the 1992 to 1994 Atlantic salmon year classes in Norway, Ireland and Scotland, but the 1999 year class in Norway was particularly badly affected, with up to 60% of some groups downgraded at harvest because of deformities (Gil-Martens *et al.* 2005). There was a large variation of incidence between and within farms, and the S0 cohorts (photomanipulated smolts) were the worst affected of all. Deformities were predominantly due to the presence of short tails, and Gil-Martens *et al.* (2005) found 13.3% of the population they studied to be suffering from this condition. No problem was apparent until several months after sea water transfer. The situation has subsequently improved significantly in Scotland and Norway, with downgrade levels falling to around 5% of harvest fish in 2004 (T. Turnbull pers. com.).

Aetiology of skeletal tissue deformities

Causes of skeletal deformities are often not entirely clear, but some factors have become apparent, such as phosphorous deficiency as a cause of screamer disease, and incubation temperature in the case of pug nose and shortened opercula in salmonids.

Vertebral deformities have also been associated with high incubation temperatures in a number of species (Wiegand *et al.* 1989, Wang & Tsai 2000, Sfakianakis *et al.* 2004) and Atlantic salmon are no exception (Wargelius *et al.* 2005). Baeverfjord *et al.* (1999) have shown a relationship between spinal deformities in Atlantic salmon and egg incubation temperature to first feeding, with around 10% of fish from batches of eggs incubated at 10°C showing radiographic evidence of spinal deformity at 60–80 g, compared to 0% in the control group incubated at 8°C. However, unlike with the incidence of some of the soft tissue deformities described above, reduction of the incubation temperature to below 8°C has not eliminated the problem outside the laboratory. Further work by Baeverfjord & Wibe (2003) has shown that the temperatures used for growing fish from first feeding are also relevant to the incidence of spinal deformity. In trials where groups of fish were grown from first feeding to 60 g at 12, 14, 16 and 18°C, the levels of spinal deformity detected in the groups at 60 g were 2, 13, 15 and 22% respectively. From this work they make a recommendation that the temperature used for growing fish from first feeding to 60 g should be maintained below 12°C. Spinal lesions which developed during this work were very difficult to see grossly, so radiographs were necessary to detect the early changes at 60 g. However, lesions of the type seen, even if very slight, have been shown to be capable of progressing once the fish are in sea water, with the potential for causing serious deformity (Witten *et al.* 2006).

Although the recommendations on temperature used for egg incubation and growth of fish to smoltification have been widely adopted by the salmonid industry, incidence of spinal deformity still persists, albeit at lower levels. Consequently work is continuing in an attempt to determine what is responsible for the continuing low level incidence.

Numerous other factors have been recorded as being associated with spinal deformity in salmonids including: specific toxins (e.g. trifluralin: Wells & Cowan 1982) and treatments (e.g. oxytetracycline: Toften & Jobling 1996), specific infections (Madsen *et al*. 2001), specific vitamin and mineral deficiencies (e.g. vitamin C deficiency: Halver & Hardy 1994; phosphorous deficiency: Baeverfjord *et al*. 1998b).

An inherited susceptibility to spinal deformity has also been demonstrated in tilapia (*Oreochromis niloticus*, Mair 1992) and Atlantic salmon (McKay & Gjerde 1986). Although the actual cause of the humpback deformity in the case studied by McKay & Gjerde (1986) was unknown – it was thought to be a nutritional deficiency or exposure to a pesticide – there was a clear genetic susceptibility to the problem. Genetic manipulation may also predispose to spinal deformities (e.g. triploidy: Madsen *et al*. 2000, Sadler *et al*. 2001). Risk factors contributing to spinal deformity have also been identified, such as timing of vaccination, type of site where fish are held, and growth rate (Vøgsholm & Djupvik 1998).

A wide range of spinal pathologies have been described associated with vertebral deformities, including: fractures (including healing and calcification); displacements (subluxation); fusion; shortening/compression (platyspondyly); remodelling/deformation; and osteosclerosis. These descriptions might indicate that numerous problems with a variety of causes have been described, or that there has been inconsistency in both pathological description and interpretation. However, it is also possible that a single primary event could lead to the variety of skeletal malformations seen (Witten *et al*. 2005).

Witten *et al*. (2005) looked at the problem of spinal deformities in the 1999 year class of Atlantic salmon in Norway, concentrating on a uniform short tail phenotype, where there was a significantly shorter overall body length than normal, but with the spine largely straight. Shortening of vertebral bodies and a reduction in the width of intervertebral spaces was found throughout the entire vertebral column in the affected fish studied. However, the calcium, phosphorous and magnesium content of the vertebral bodies from affected fish were identical to those from normal fish. The spongy bone of vertebral bodies was also normal in appearance. The main changes were a structural alteration in the vertebral end plate growth zones, alteration to intervertebral tissue, and replacement of notochord by cartilaginous connective tissue with evidence of calcification, but with notochord tissue still present centrally. None of these changes were associated with significant inflammation, as found in previous studies (Kvellestad *et al*. 2000, Madsen *et al*. 2000).

The conclusion from this work was that the problem being examined was related to a relatively late stage lesion, with structural alterations in the growth zones leading to alterations in the shape of the resultant vertebrae (Figure 13.1). It was speculated that notochord damage may have initiated the changes. The notochord has a known role in vertebral development and, although it transforms to cartilaginous tissue in higher vertebrates but persists as notochord in fish, it seems likely that its developmental role persists (Grotmol *et al*. 2003, 2005). Mechanical force can initiate cartilaginous change in notochord (Lotz *et al*. 2002), and Witten *et al*.

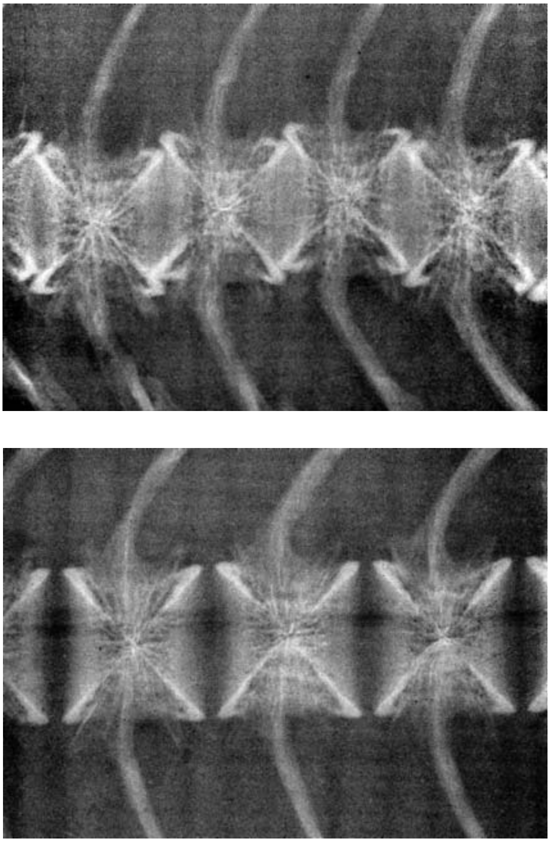
(a)
(b)


Figure 13.1 (a) Vertebrae from short tail Atlantic salmon showing structural alterations in the growth zones, compared to (b) vertebrae from a normal fish.

(2005) suggest that mechanical overload during husbandry operations could be a possible cause of this initiating damage.

Other work has shown that vertebral fusion may also be associated with the short tail condition (Gill & Fisk 1966, Kvellstadt *et al*. 2000), and a further study by Witten *et al*. (2006) suggests that fusion might also be related to mechanical influences on the notochord structure, and that it can occur at any stage during the life of the fish.

So, the causes of vertebral deformities in Atlantic salmon, and presumably other fish species, can be complex. Clearly factors such as temperature in the early stages of life are important, as can be, for example, diet and exposure to toxins and infectious agents, but other factors, many of which have not yet been determined, are also clearly involved, and more research into the subject is needed. Probably the most important work for welfare purposes is the determination of exactly when, and under what conditions, each deformity develops. With this information, and the knowledge that deformities may be initiated at any time during the fish's life, probably associated with mechanical trauma, then avoidance strategies may be

devised. In addition, Witten *et al*. (2006) have shown that vertebral body damage can be contained by the fish, with successful remodelling of fused vertebra, thus minimising the effects of vertebral damage. However, to allow this containment to take place it will be necessary to know which developmental stages and farming conditions favour the containment, and which will aggravate the problem and lead to further deformity.

Conclusions

Many deformities of fish have been recorded, in wild fish as well as those in aquaculture, and most will have some impact on welfare. The causes of all these problems may be, and probably almost certainly are, complex. Some are incidental, and have little or no impact on fish or farmer, but some can be very costly in both welfare and financial terms.

In the case of deformities which have an impact on welfare, whatever the level of incidence, their presence will raise the ethical question of whether or not aquaculture should continue if fish cannot be produced without significant levels of deformity occurring. However, it should be remembered that many of the deformities seen in larger fish are the natural product of a population which is not exposed to natural selection pressures, resulting in many fish being kept alive in aquaculture which would normally die or be predated in the wild. This will result in an unnaturally high level of 'natural' deformities, and could support an argument in favour of continuous grading and culling of affected fish. But there are also undoubtedly factors occurring in aquaculture which can, and do, contribute to these problems, and some of these will be inevitable as production methods change within what is actually a relatively new farming venture.

However, the research effort being invested into causes of deformity in aquaculture is considerable, and the aquaculture industry has been quick to adopt corrective measures as soon as these measures have been defined, as has been demonstrated by the significant improvements seen in the incidence of vertebral deformities in farmed salmon over recent years. More work is clearly needed though, and is part of a continuing process.

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Chapter 14
Welfare of Fish at Harvest

David H.F. Robb

Introduction

Harvest comes at the end of the production cycle of all animals farmed for food. With fish, there are numerous different procedures used, depending on species, equipment, location and degree of awareness or training of the operators. The variety is quite overwhelming, even for a single species, but general themes can be followed throughout the process. During the process, a number of actions are implemented which can or do affect welfare. Some are avoidable, but others are not. For instance, it could be regarded that actually killing the animal is the greatest insult to its welfare – but it is of course unavoidable in a harvest procedure.

The generalised harvest procedure is outlined in Figure 14.1. As mentioned above, there are many different variations to this, but it will provide a structure to this review, following through the process running from the end of normal production (growth of the fish) to ending up with a dead fish. From this point onwards, the welfare of the animal can no longer be compromised.

This review will comprise a mix of published papers and articles with personal observations and anecdotes. It is based on the current commercial methods used to kill salmonids, although where there is information, other species are included. This is due mainly to the scarcity of published material on many individual aspects of fish harvesting. A restriction on the amount of available literature comes from the complexity of carrying out research on the commercial methods to a degree which is suitable for publication as a scientific paper in a journal. This is coupled with the only relatively recently developed interest in harvest methods. There is still a lot to learn about pre-harvest and harvest methods and scope to develop more systems which promote good welfare at this important stage.

Pre-harvest preparations

Harvests should be planned operations, following a production strategy to grow the fish to a certain marketable size. Sometimes, due to unforeseen circumstances, most noticeably disease striking a farm, fish have to be killed earlier than expected.

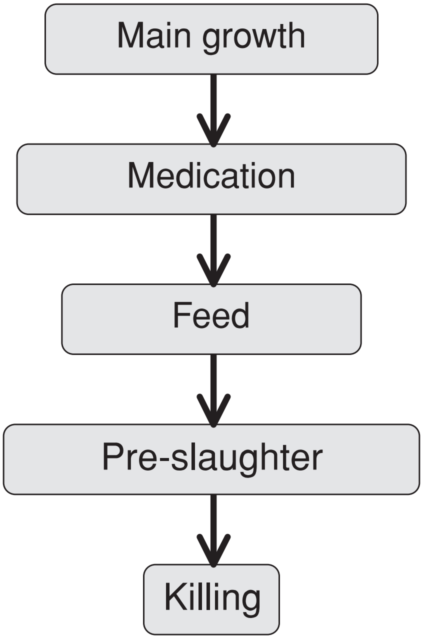

Figure 14.1 A generalised harvest scheme for farmed fish.

However, mainly there is a period of preparation in the time coming up to harvest, which is where welfare concerns related to harvest will first arise.

Medication withdrawal

Veterinary treatments of fish are covered by legal licence. This is issued after determination of a variety of issues, including the food safety impact of any residues in the flesh and how long it takes the fish to clear these residues during life. This leads to a designated maximum residue limit (MRL) in the flesh and a withdrawal period which is required in order to achieve that MRL. The withdrawal period is species dependent and is often temperature dependent too, fish being poikilothermic.

Back-calculation from the expected harvest date, using knowledge of expected temperatures, allows the farmers and veterinarians to determine the last possible date of treatment. Beyond this point, the fish may not be treated for food safety reasons. Consequently, a problem arises if a disease requiring treatment occurs after this last possible date for treatment. This could be a new disease, or a recurrence of a disease already treated. As medicine residues are cleared from the flesh, the fish becomes less resistant to the diseases the medicines were designed to protect against. The clearance of the medicine from the flesh will be logarithmic, dropping rapidly at first and then more slowly as the concentration reduces. Therefore the fish will rapidly lose protection once treatment ceases.

Some medicines only have short withdrawal periods – a few days – while others may require months. During such long periods, if infection occurs, then a decision has to be made as to whether to treat the disease and start the withdrawal period, or to keep going without treatment. It may become a race between getting the fish to harvest weight and serious disease occurring. Not treating is likely to impinge on the welfare of the fish which are diseased, but starting treatment may also have an effect. It will be longer before the fish can be harvested, so they may become overcrowded in the enclosure, leading to other stresses. Or the fish may start to mature. Balances have to be struck against commercial consequences of not harvesting the fish at the planned time, and the risk of all the fish becoming diseased if they are not treated. There is no simple answer to this and the decisions have to be taken on a case-by-case basis by the local veterinarian.

Some codes of conduct greatly increase this risk, by demanding longer withdrawal periods than the legislation – maybe even doubling the time. In the case of UK legislation, for example, where the MRLs have been calculated on a highly scientific and conservative basis, this makes no sense at all in terms of food safety, but does greatly increase the exposure of the fish to risk of infection and consequently potentially compromises their welfare. Such codes tend not to be based on any science, but more on a market position and a need to appear to be different. In reality, the food safety of the product is likely to be very little improved.

Pre-harvest feed withdrawal

The second major pre-harvest procedure is the withdrawal of feed from the fish at some point before the time of slaughter. There are various thoughts behind what feed withdrawal may achieve, but research has supported few of these. In the past, long withdrawal periods have been implemented in the hope of: emptying the intestines of food and faeces for food safety reasons during processing; reducing fish flesh fat content; improving the texture of the fish (Einen *et al.* 1999); and removing any flavours carried over from the feed which may be detrimental to the fish flavour. The period could last from a few days to over a month.

In practice, it has not been demonstrated that there is any significant loss of fat (in percent of muscle weight) even over extended periods of feed withdrawal (Einen *et al.* 1998, Einen & Thomassen 1998). To address the points relating to flavour and texture, at least two commercial trials have been carried out (unpublished) where fish were starved for a period between 0 and 14 days and were then cooked and eaten by internal taste panels (in other words not trained or selected panels, but people from the companies who regularly screen the fish for flavour and texture to check that no irregularities occur). In both trials, the groups most preferred by the panellists were the fish which were not starved at all. If the fish do contain any 'feed' flavours, it would appear that these were considered positive attributes by these taste panellists.

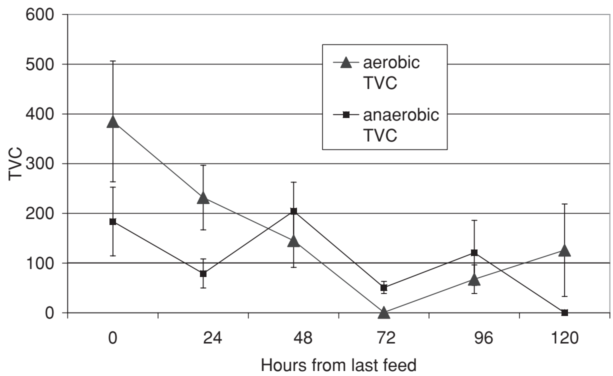

Figure 14.2 Total viable counts (TVCs) from Atlantic salmon viscera after a period of feed withdrawal (A. Smart pers. com.).

Feed withdrawal does have an important role in pre-harvest management. By removing the feed, the excretion of ammonium by the fish is greatly reduced. This is particularly important in systems where the fish are transported live, as this reduces the rate of water quality deterioration. Feed withdrawal also reduces the number of bacteria in the gut over the first few days (A. Smart, Smart Aqua pers. com.). Interestingly, after about three days of feed withdrawal, the bacterial populations reach their lowest and start to increase again, changing types (Figure 14.2). This supports the need to keep these periods fairly short.

Under farming conditions, fish are presented with feed regularly, either as set rations at times over the day or 'to appetite' through the use of equipment which can detect whether the fish are feeding or not – if they are eating the food, more is given to the cage, if not the feeding is stopped. An individual fish may not eat every day – but this is due to its choice. In the wild, fish may go for long periods of time without food, but this is not by choice (except at the final migration upstream, when their body is preconditioned to starving) and they have the opportunity to look for food. In a farm system they have no chance to look for food. While there is no published information to show whether or not food withdrawal at harvest is adverse to the welfare of the fish or not, long-term food withdrawal will certainly reduce the levels of readily available energy (e.g. liver glycogen as shown by Wiseman (1993)). This makes the fish more susceptible to stress and disease, coupled with the medication withdrawal, mentioned above.

The only demonstrated effects of a pre-harvest starve are the emptying of the viscera of food and faeces, reduction of intestinal bacteria loading and the reduction of ammonia excretion by the fish. The empty gut is necessary for commercial processing from a food safety point of view, as a full gastrointestinal tract is very likely to become punctured during evisceration, which will result in gut enzymes and potential pathogens being spread on the flesh. The reduction in visceral bacteria over the first three days also assists with the food safety requirements (A. Smart

pers. com.). Gut clearance is a time and temperature dependent equation, which requires a longer time at low temperatures, even though the fish may be eating less (Usher *et al.* 1991). However, independent of the temperature, the process is complete by three days in Atlantic salmon, *Salmo salar*. This sets the minimum starve period.

For large cages, it may take several days, or even over a week to harvest all of the fish. As it is not possible to feed some fish in the cage whilst starving others, the last fish out of the cage will have been starved longer than the first. Although there is no evidence to show if or when this would become a welfare insult, there must be an impact with increasing time – the reduced readily available energy reserves for instance, which could reduce the ability of the fish to cope with any additional stressors imposed. Therefore a maximum time is debated, beyond which the harvesting from a cage should stop and the surviving fish be returned to feed. In the past, very long starve periods, even extending over a month, could be encountered – these are clearly unacceptable. Certain standards are now stipulating a maximum of 14 days, while others are down to a 5-day maximum. The latter give strong constraints on some commercial sites and risk over-run if bad weather prevents harvesting as planned. However, the overall concept has been accepted that long starve periods achieve nothing and so starve periods have generally been reduced.

This also leads up to the debate on what happens when the maximum starve period is reached. Under the codes of practice, if the fish are not already harvested when the maximum time is reached, they should be returned to feed. Again there is no published data to show how long they should be back on feed before the next starve period, with respect to welfare or any other parameter. Current practices run between two and four weeks before the next pre-harvest starve is imposed, but this is not based on any scientific information.

With the viscera cleared of food and faeces, the fish are now ready for commercial harvest. A wide variety of handling procedures can occur from this point on (Figure 14.3).

Crowding

In every aquaculture system, the fish are raised at a stocking density at which it is not economically viable to spend effort on catching the fish to remove them from their tank, pond or cage. This density has to be increased in order to facilitate their removal and this is known as crowding. This is the first stage of the pre-harvest handling. Crowding is certainly stressful to the fish to some degree (Erikson *et al.* 1999, Skjervold *et al.* 1999, 2001), but the methods used to carry it out can be managed to minimise this stress.

Crowding can be achieved in a variety of ways, depending on the system in which the fish are contained. In tanks or ponds, the water level can be dropped,

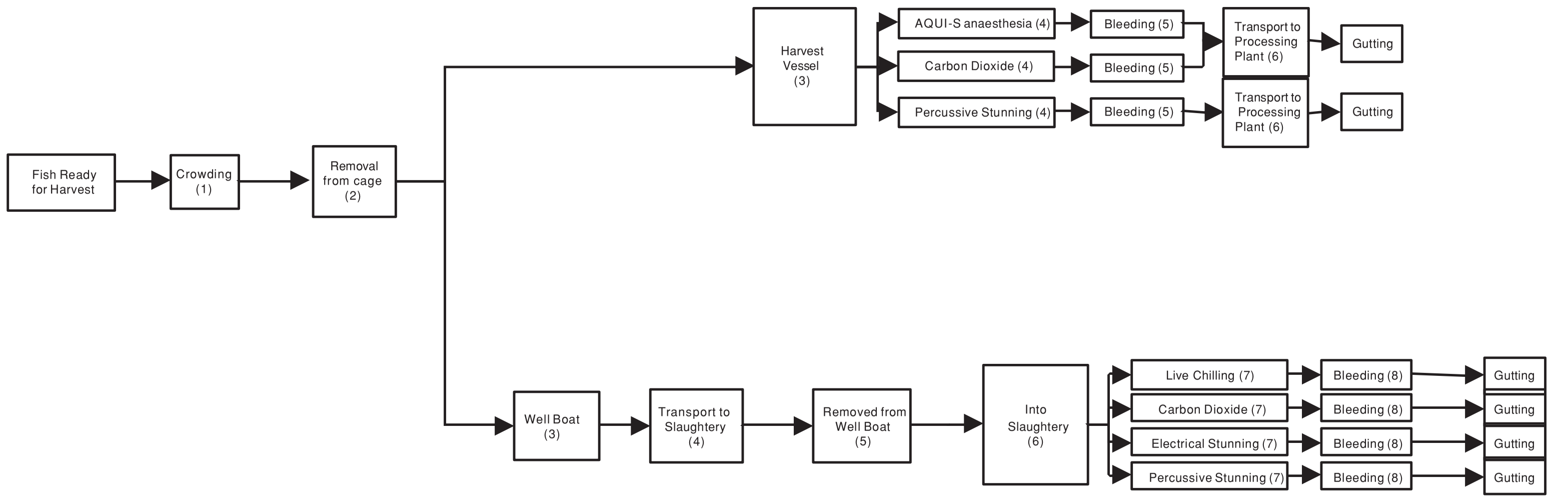

Figure 14.3 The number of possible steps in the handling and processing of fish, once starved ready for slaughter.

concentrating the fish at the bottom. From here they can be chased into a smaller area, often using nets or barriers. In some pond systems and in cages it is not possible to drop the water level and so an alternative has to be used. In cages, the main cage net can be raised, crowding the whole population, or for both ponds and cages a sweep net can be used, crowding all or just a part of the population depending on how it is set.

A sweep net is a small seine net, pulled through the cage to concentrate the fish. A corked line keeps the top edge of the net at the surface. The remainder of the net hangs down through the water, pulled down by a lead line, and the whole net is pulled through the pond or cage by ropes at the four corners (top and bottom). Control during the process reduces the chance of fish escaping round or under the net. This normally involves pulling the net slowly rather than quickly, which lifts it up and allows fish to escape. The slow movement of the net also stresses the fish less, compared to rapid movements which make them swim rapidly away.

A variety of commercial investigations have been made into the different nets which can be used for sweeping. There is some consensus. The net should be soft, as it does not have to survive long periods in the water with fouling growing on it, and must not damage the fish at harvest. This helps to keep the fish calm as it is not abrasive if they come into contact with it. It also helps the net hang down in the water, facilitating the catching process. The sweep net should also be relatively large compared to the cage or pond in which it is used (K. Rzepkowski, Grading Systems pers. com.). If the sweep net only just reaches across the cage, it has to be pulled out taut making it harder to use and more stressful on the fish. A much larger net can be allowed to bag, which keeps the fish calm and reduces damage to them if they swim into it. It is also much easier to set, apart from the extra weight, as it can be pulled along the edge of the pond or cage without fish easily swimming round it. A bag shape rather than a flat panel also helps with this, encouraging the fish to swim to the centre of the bag.

The colour of the net is much debated and a variety of trials have been carried out. Black nets are harder for the fish to see and there is an idea that this may stress them less. However, it is hard for the operators to see the fish against them, so it is difficult to gauge how many fish are caught in the sweep. Too many and fish will be unnecessarily stressed in the crowd, as they will not be harvested that day. Too few and another crowd will have to be set, wasting time and effort and possibly stressing more fish. Therefore white nets were also tried, but some operators felt the fish were avoiding these and were harder to catch. This led to a black net with a white border being tried. This whole debate was also affected by different fish stocks – some strains seem to prefer being deeper in the water than others and would readily dive under white nets which were easy to see (B. Beens, Mainstream Canada pers. com.). There is certainly no consensus on net colour and it seems to be driven by the operator's personal choice. However, there is probably an effect of net colour which should be investigated – it will almost certainly interact with the amount of light at the time of crowding (see below).

In square cages it is also possible to crowd fish, either the whole population or a portion, by lifting the nets. The fish are crowded towards one side of the cage, rolling the nets up gently so as not to make sudden movements which will cause stress. A split of the population can be achieved by lifting up the cage across the middle, and then crowding half – the remainder stay uncrowded in deep water and so seem unstressed. This creates a channel of more densely crowded fish at one side of the cage, which can be further split, giving a working area of densely crowded fish ready to be removed from the cage. Such a method keeps the time any individual fish is tightly crowded to a minimum, maybe as low as 10 minutes if there is a good system, but this will require a lot of personnel to arrange (pers. obs.). Lifting the nets to crowd the fish is much more difficult in circular cages, where a sweep net is the best option for practicalities and for the fish.

Having crowded the fish, the high density is then maintained during their removal from the cage. The density of the fish will affect the degree of stress they experience (Skjervold *et al.* 1999, 2001). However, duration and environmental factors will also modulate the stress. Some of these may be controlled, others are not controllable and therefore must be worked around in order to minimise the stress.

Crowd duration has an effect on the stress felt by the fish. Atlantic salmon maintained under similar conditions throughout two commercial harvests were studied and blood sampled (D. Robb, unpublished data). In each case, the fish were crowded to approximately the same degree, but in the first harvest the fish were taken in one large crowd and held for a total of four hours until the last fish was killed. In the second harvest, two consecutive sweeps of approximately the same size were made, each lasting just over one hour (the killing rate in the second harvest was faster, but the crowd density appeared to be similar as did the reactions by the fish). Blood samples taken from fish chosen at random throughout the harvests showed a trend for plasma cortisol to increase throughout the crowd period (Figure 14.4). However, in the second harvest, the fish taken in the second sweep only

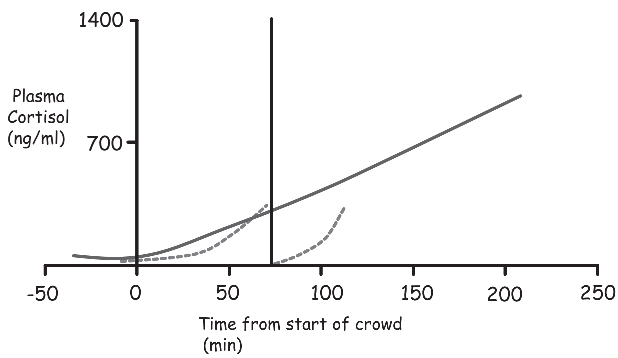

Figure 14.4 Trends of plasma cortisol concentrations to rise during two different harvests. The first took all of the fish in one large sweep (solid line), while the second harvest used two sweeps (dashed lines).

experienced the same low level of stress experienced by those in the first sweep. Therefore, the fish in the second half of this harvest were much less stressed than those in the second half of the first harvest. This shows that management practices which keep crowd durations short, such as using two sweeps instead of one or fast removal of the fish from the crowd, will help to reduce the stress imposed on the fish by the system.

The amount of ambient light at the time of harvest has a big effect on the degree of stress experienced by the fish. Salmon prefer to avoid bright light, keeping down in the cage on bright sunny days (pers. obs.). However, during crowding, they are brought up to the surface. On a sunny day, the fish will try to escape, thrashing vigorously at the surface, while they will be much calmer on a cloudy day given the same conditions. At night, the salmon are even quieter, often being up at the surface of their own volition before the crowding starts (pers. obs.). Although it is apparently less stressful to work with the fish at night, there are health and safety concerns for the operators with regards to this. Also, at night the oxygen concentration in the water is often lower, increasing the potential for fish stress – a difficult balance.

The amount of light the fish are exposed to determines the optimum shape in which the net should hang in the water. In order to allow the fish to be away from the light and activity at the surface, it is important to have a deep net pocket. If the nets are pulled up tight to the surface, the fish are brought up to the light and human activity which results in a lot of escape behaviour. A side effect of this is that when nets are brought up to create a shallow net pocket, they are very taut and abrasive. When the fish rub against them, they easily abrade scales and quickly become damaged, which aggravates the stress. In contrast, a net with a deep pocket hangs loose in the water, so that when fish move against it, there is very little resistance, so minimising the damage and stress. The deep net pocket also allows fish to move to the darker, cooler water if they desire. With fish crowded to about the same density in the two types of net pockets, there is much less underwater and surface activity in the deep net pocket. However, as the density can be the same in both, it is just as easy to remove the fish from the deep net pocket set up (pers. obs.).

Water quality is always important to fish: the water provides oxygen and removes the waste products carbon dioxide and ammonia. When fish become stressed and more active, they use up more oxygen and produce more waste. This in turn adds to the stress. When fish are crowded at high densities, there is less oxygen available and the concentration of waste products will increase as there is less water per unit fish within the crowd. This is often compounded by reduced water flow through the volume occupied by the fish during the crowding – often the main nets of cages are fouled with plant and animal growth at the end of the production cycle. The longer the fish are in such conditions, the worse the stress will be, though the time limits will be species dependent, as some species are much more tolerant of poor water quality than others.

Practically, it is not possible to reduce ammonia or carbon dioxide concentrations in the water, other than by increasing water flow or reducing fish densities. Ammonia

concentration is managed to a certain extent by feed withdrawal some days prior to crowding (see above). Oxygen can be added by bubbling oxygen or air into the water through diffusers. This can be very effective at increasing oxygen concentrations in the water in the crowd, calming the fish (pers. obs.). The oxygen concentration in the water in the crowd can also be assisted by ensuring that the nets around the fish – both the main net and any sweep nets used – are clean and free from fouling.

The oxygen concentration in the water can also be increased by pumping water into the cage. If the water from outside the cage is of good quality – which must be checked before starting – the fish will then move to the area around the inflow pipe.

Both the addition of fresh water or oxygen encourage the fish to come to a certain point within the crowd and this can be used to assist the next stage of harvesting – the removal of the fish from the cage. This is where the fish are taken out of the cage and to the next stage of harvesting, and also where an incredible amount of variation in the methods of handling starts!

Removal of the fish from the cage

The oldest method of removing fish from water is to net them out. This can be done by hand, using a small net. This is still essential for removal of casualty fish – fish overly stressed by the crowding process, which are moribund at the surface and should be taken to casualty slaughter immediately (see later section) – but for larger harvests this method has now been largely discontinued. Hand netting has instead been mechanised with the use of a brail net. A metal hoop, about 1 m in diameter, has a net tube hung from it, with the free end attached to a rope which allows this end to be opened or closed. The hoop is suspended from a small crane, which is used to drag the net through the crowded fish, catching them. The net can then be swung to where the fish are required, the end is released using the rope and the fish flow out. There are a variety of methods of doing this. Most fix the net almost to the end of the crane and use the crane arm to drag the net across the surface of the crowd. This requires the fish to be in a shallow net pocket – or they all dive down in the cage and escape – and can be very stressful on the fish (see section above). An alternative requires a small winch on the crane. The net is then suspended from a rope, allowing it to be dropped to the bottom of the crowd. A deep net pocket with a narrow width – which results in relatively low stress conditions for the fish – is then used. The net is winched rapidly to the surface, catching fish on the way. This method requires a bit more equipment than the previous method and is much less common, although it is a better method for Atlantic salmon, as it enables the deep net pocket crowd to be used (pers. obs.).

Both methods of brailling result in the fish being removed from water and struggling in the net. With a high density of fish in the brail net, a lot of damage can be done to the fish, which is bad for welfare and fish quality. To reduce this, a tarpaulin

liner can be added inside the brail net. This retains some water with the fish and separates them from the abrasive net. Although this is a better solution, there is still a degree of stress in the catching. However, the main stress of brailling comes through overloading the net. Tight crowding in the main cage coupled with poor brail operation can result in very high densities of fish being caught in the brail net. Even with a tarpaulin liner, there is very little water and the fish very quickly become stressed and struggle intensely. With operator training, the number of fish caught can be reduced, but this then becomes relatively slow and difficult to work under commercial situations.

Pumps are routinely used throughout the salmon industry to move fish from the crowd to another place – typically either to a killing table or to a well boat (see later sections). There are various types of pump set ups which are used, which have various advantages and limitations.

Air lift pumps require the least specialist equipment, but are more difficult to set up and run efficiently. A compressor is used to blow air down a pipe, which runs underwater. This is connected to a vertical pipe, so that the air bubbles back up to the surface. As the air rises rapidly, it can draw more water in, through a side pipe, which runs from the crowd, sucking up fish at the same time. If the air can be pumped down to sufficient depth, and the pump delivers enough volume of air, water and fish can be lifted several metres up above the surface, allowing them to be delivered onto a boat or working platform. These pumps are good for moving fish short distances, steadily and in water at all times. However, care needs to be taken with the set up – some sites are not suitable as insufficient depth is available for pumping the air down and often the compressor cannot deliver enough volume of air to achieve an efficient operation.

Venturi pumps force a large volume of water at speed through a pipe, drawing up water and fish from a second pipe placed in the crowd. The pumps can have good suction, moving fish quickly through the system. But they also deliver a lot of water with the fish, which has to be separated. This can cause problems if there is not a good drainage system.

Vacuum pumps suck water and fish up from the crowd and into a chamber through one pipe and then blow them out of another pipe. The system creates a strong suction and care has to be taken that the vacuum chamber is not much higher than the water containing the fish, or the fish will suffer from damage. This would be seen as haemorrhages internally and burst eyes externally. In practice, the pumps are normally situated about 2 m at most above the fish. The power of the pump can then blow the fish up several metres, without apparent damage, allowing them to be delivered into boats or factories if required (pers. obs.).

Unlike the other pumping systems, vacuum pumps do not deliver a continuous flow. Fish move through the system with stops and starts as the vacuum chamber loads and discharges. The timing of cycles can be controlled manually, but as the cycle shortens fewer fish are caught, so the temptation is to use long cycles. However, if there are a lot of fish in the chamber, they tend to struggle and use up

their oxygen supply, so increasing the stress. The power of the pump also allows for fish to be pumped over long distances – several hundred metres if required. This takes a long time and fish may be in such a system for over 10 minutes (pers. obs.). In this scenario, the fish are in relatively little water with decreasing water quality, in terms of oxygen and possibly increasing temperature too if it is a sunny day. In order to remain in control of the water quality, the pump distance and the time any one fish spends in the pipe must be short. This is best determined by monitoring the change in water quality between intake and outflow under 'worst case' conditions – highest ambient temperatures are likely to cause the largest change. If there is a significant change then this will be stressful to the fish.

After being removed from the cage, the fish can either be killed at the site, or can be transported to a harvest station, like an abattoir. As the killing methods are similar either on site or at a harvest station, the next section will deal with transport to slaughter.

Transport to slaughter

Well boats are the main method of transporting live salmon to slaughter. Able to move easily from site to site between farms and then on to the slaughter station, they can also carry large volumes of fish, making them cost effective. In countries such as Norway, they are considered essential to the industry, as farm sites are often far from land-based processing facilities. As such, journey time may range from less than an hour to almost 30 hours, depending on the circumstances (Iversen *et al.* 2005). During this time, it is essential to maintain a minimum water quality in order to keep the fish alive and unstressed (Erikson *et al.* 1997).

There has been an evolution in well boat design to assist with the capacity to carry large volumes of fish. Initially, well boats were basically large, floating containers, holding a set volume of water in which the fish were held. However, this limited the volume of fish which could be transported, as the fish would use up the oxygen in this water. This led to the development of so-called open-hold systems. Water could be pumped into the holds during the journey, flow past the fish and out of the stern. In this way, the fish received oxygen from the incoming water and their wastes were washed away in the outflow. For a period of time this was the acceptable method of transport.

Well boats were implicated in the spread of disease between sites (Anon 2000a). The outflow water from the fish being transported was identified as a serious risk to farms which the boat passed. Codes of practice were drawn up to reduce this risk (e.g. Anon 2000b). Boats were not to run on open-holds as they passed other farms. For instance, in the UK, the code of conduct states that boats should be closed-hold when they are within 5 km of another site. This restricted the access of the fish in the hold to oxygen again. Oxygen diffusers were then added to the boats, so that

the concentration of dissolved oxygen in the well could be maintained. At first the monitoring and supplementation of the oxygen was carried out manually, but then efficient automatic systems were developed. However, although oxygen could be added to the water, carbon dioxide and ammonia concentrations increased as there was no control over these waste products. This, coupled with any fluctuation of the oxygen control would result in heavy stress or mortality to the fish during transport.

This led to the development of the latest generation of well boats, boats designed to keep fish for extended periods under closed-hold conditions. Carbon dioxide concentrations are reduced by intensive aeration of the water. This can be done by spraying the water onto a metal plate in the air and returning the water back to the fish, or by forcing the water through a raceway where a lot of turbulence and foam is created. The increased aeration displaces the carbon dioxide as well as adding oxygen. It also creates a froth of protein, removing this from the well where it may irritate and stress the fish. The carbon dioxide control is also coupled with temperature control. Fish may be slowly cooled within the wells by on-board cooling units. Fish are chilled from ambient to the desired temperature at a rate of about 1°C per hour, which also is beneficial for the packing station after killing. Chilling the fish slowly does not appear to be stressful to the fish (pers. obs.), but will reduce their metabolic rate, so reducing their waste production. This is different to the rapid live chilling method described below.

The impact of well boats on the welfare of the fish is poorly understood, mainly due to the different types of boats in use and their various likely effects on the fish. Boats operating closed-holds for extended periods without good water quality control certainly have an impact on the fish (Iversen *et al.* 2005). Although the fish may appear quiet, they can be very stressed within the wells, becoming either very dark skinned or very pale. Mortalities have been associated with well boat transports – even entire shipments have been lost due to poor water quality control. However, if good water quality is maintained, well boat transports may be considered welfare neutral: they neither improve nor reduce the state of the fish. The potential stressors during well boat transfer then become the upload and offload. But it must be noted that with current restrictions on well boats having to operate closed-holds as they pass sites, not all well boats are capable of maintaining the water quality at a welfare neutral state (J. Avizienius, RSPCA pers. com.).

Offloading of well boats is normally done by pumping the fish out of the wells, either to a holding cage or directly in the harvest station. As the number of fish in the well decreases, the water level is dropped to crowd them. At the end, the water is almost completely removed and the last fish are pushed into the pipe. This low water level is certainly stressful for the last fish and may be before this point too, depending on how quickly the water is lowered (Erikson *et al.* 1999). An alternative to this is to use a moving bulkhead in the well. As fish are removed from the well, the bulkhead is slowly moved up, so keeping the fish crowded. By observing the fish, either directly or by camera, the operators are able to minimise stress to the fish

in this procedure. Using this method, the last fish can be removed without lowering the water levels or causing further stress.

Offloading may be direct into a shore-based slaughter unit, or to a holding cage immediately next to such a place. Holding cages are popular, as they allow the well boat to move off to collect the next load without having to wait. They also maintain a constant supply of fish to the slaughter plant, even if there is bad weather which disrupts the transports. However, in order to survive the holding period, the fish have to be in good condition following the transport. Badly controlled transport and unloading have been responsible for high mortalities following smolt transports and also harvest transports (Iversen *et al.* 1998). If the fish are to be held in such cages, the water quality at these sites must be suitable – sites in sheltered areas, where there is poor water circulation, will be stressful to the fish. Holding cages also have the requirement to crowd the fish again before they can be removed and killed. This leads to the possibility of a build up of stress within the fish over a few days. If they are to be welfare neutral, the conditions at these cages must be very well managed.

Sedation, stunning, slaughter and killing

Following removal from the cage or well boat, the fish are now ready for killing. They may be stunned – either electrically or percussively – or put through a system which 'sedates' them, or at least ensures that they are quiet when they come to be killed, which is normally by exsanguination.

At this point various terms are used loosely, both commercially and within the scientific literature. In order to avoid confusion, a short description of some of the terms as used in this chapter follow.

Stunning is a process which causes immediate loss of consciousness, which should last until death (Anon 1995a). *Narcosis* is a process which renders an animal immobile. *Analgesia* is a process which renders an animal insensible to pain, although it may be aware of other external stimuli – so it may be able to see for instance. *Anaesthesia* is the combination of analgesia and narcosis, so the animal is immobile and insensible to pain. *Slaughter* is the process of killing an animal by *bleed out* (Anon 1995a): the animal will die of anoxia in the brain through lack of blood. Commercially for example, an animal may be stunned (rendered immediately unconscious) and then slaughtered (bled by cutting a major blood vessel, which will actually kill it). From a welfare point of view, the process of loss of consciousness should either be immediate, or should not induce any stress. Also, no fully or partly conscious animal should be bled, as this is also stressful.

Various methods are used to assess the processes used. At the most basic level, studies of the behaviour can be used. Kestin *et al.* (2002) developed a scoring system using swimming ability, reactions to touch, gill ventilation and eye control by the fish to indicate the degree of awareness. This was validated in part by correlation

with direct measurements of brain activity in response to external stimuli. Kestin *et al.* (1991, 1995) and Robb *et al.* (2000) measured the responses in the brain to flashes of light in the eye (so-called visual evoked responses, or VERs) to determine the basic ability of the brain to process external stimuli. Lambooij *et al.* (2002a) applied direct tactile or electrical stimuli to the fish to elicit a pain response in the brain which could be measured (so-called somatosensory evoked responses or SERs). Lambooij *et al.* (2002b, 2003, 2006) also looked at changes in the waveform of general brain activity to detect the level of responsiveness. Using fast Fourier transformation of the detected brain activity, the frequencies of electrical impulses within the brain can be determined and interpreted to show levels of awareness.

The measurements of brain activity are slow and relatively difficult to carry out, so there is a general reliance on visual assessments to determine the efficacy of killing systems. Blood chemistry responses tend not to be sensitive enough (e.g. cortisol) or too variable either within or between experiments (e.g. adrenalin) to be useful for assessing the killing stage (Pottinger 2001). However, blood lactate, or muscle pH, may be used with care as indicators of muscle activity (which could be an indicator of stress). Care needs to be taken in using these that all fish are handled in the same way before the killing method is applied. Control samples also are required to determine the state of the fish before starting the killing method – if they are already highly stressed, it is likely that little difference will be detected between the killing methods using blood chemical analyses. This is where behavioural observations become an essential tool, complementary to the chemical analyses, for interpreting whether systems are stressful or not.

Carbon dioxide narcosis

Carbon dioxide is routinely used in the commercial slaughter of various different food animals including Atlantic salmon, rainbow trout, chickens and pigs. It is used because animals can be dealt with in batches – often large numbers of individuals – and so requires relatively little labour. There is also a perception that it is not stressful to the animals, although this is being widely challenged now with mounting evidence against this (Conlee *et al.* 2005).

In aquaculture, carbon dioxide has been considered useful as it is relatively easily dissolved in water, creating an acidic mixture. Fish can be added to this water and left there until they stop moving. They can be removed once they are quiet enough to handle, killed by bleeding and processed (pers. obs.). This has been used for many years, lately also in conjunction with the use of chilling (see later section), especially in regions where labour is relatively expensive, as a small team can kill a large number of fish using this method.

There are various commercial recommendations for the concentration of carbon dioxide required, which basically require saturation of the water with the gas (e.g. Anon. 1995b). Generally, there is relatively little control commercially, with the gas being bubbled into the water through a diffuser, which may vary greatly in efficiency,

although the pH and oxygen concentrations may be measured (pers. obs.). With an efficient diffuser and low water temperature (which increases the amount of gas which can be dissolved), the saltwater pH will be 5.5 or lower. In most situations, the water will not be changed, or will receive some top up, throughout the harvest. Aside from the deliberately high concentration of carbon dioxide, the water will also increase in ammonia concentration and decrease in oxygen (although some practices add oxygen to the water).

On entering the carbon dioxide solution, the fish show an immediate and strong aversive reaction. Investigations into this have shown that it is not just the low oxygen or acidic conditions which cause this reaction, as exposing fish to these on their own results in a lesser reaction (S.C. Kestin unpublished results). In this study, fish were videoed on being placed into a small container of fresh water, a container of water with deoxygenated water, a container with carbon dioxide saturated water or one with water at the same pH as the carbon dioxide saturated water, but with the acidity caused by the addition of nitric acid. Video clips of the fish reactions were played to observers, who were blind to the treatments. The fish added to the carbon dioxide treatment clearly showed the strongest aversive reactions. Commercially, the reaction will not only be to the carbon dioxide solution, but to the increased ammonia in the water, as the struggling fish will excrete ammonium which will be rapidly turned to ammonia in the lower pH.

Robb *et al.* (2000) investigated the time it took for Atlantic salmon to lose VERs when exposed to carbon dioxide saturated water. The authors showed that there was no reduction of VER amplitude until there was a sudden total loss – which occurred 300 and 554 seconds after immersion in fresh water saturated with carbon dioxide. During this time, however, the fish became completely immobile – after between 2 and 3 minutes. This implied that a narcosis had occurred, although the full amplitude VER response indicated normal brain activity, reducing the likelihood that there was any analgesia. There was certainly no stunning effect meeting the requirement of Anon (1995a) – immediate loss of consciousness – nor could the process be considered low stress, as before becoming immobile, the fish had shown a high degree of activity. The long period between the cessation of activity and the loss of VERs indicates that in commercial systems, where the fish are bled as soon as they stop moving enough to be handled, the majority of fish are slaughtered when fully conscious.

The activity shown by the fish during exposure to carbon dioxide is great. Observations show this and muscle pH measurements also demonstrate the large increase in lactic acid built up during this intense swimming period. Kiessling *et al.* (2004) also demonstrated that the flesh pH of fillets stored after killing was much lower in Atlantic salmon exposed to carbon dioxide than in fish which were anaesthetised with iso-eugenol (a very gentle anaesthetic for salmonids).

The combination of intense activity with full awareness and apparent distress implies a major impact on the welfare of fish exposed to carbon dioxide. Carbon dioxide does not apply a stun under the definition of Anon (1995a). There is no

evidence of anaesthesia, although there is a narcotic effect. Commercially, it is likely that the majority of fish are bled while fully conscious. In summary, this is not a method of handling the fish which promotes good welfare.

Live chilling

As fish are poikilothermic, their metabolism can be reduced by rapidly reducing their body temperature. This results in lower muscle activity, as the muscles become apparently almost paralysed by the cold. There is also a requirement to chill fish after killing for food safety and shelf-life reasons – cold fish show slower autolysis after death and pathogenic bacteria such as *Listeria* spp. do not multiply so quickly at lower temperatures. Immersing live fish in very cold water or ice results in a more rapid chilling of the muscle (Skjervold *et al.* 2002) and so is attractive to fish processors.

Wild caught fish have been tipped directly from the nets onto ice for a long time historically and farmed rainbow trout, *Oncorhynchus mykiss*, have also been killed in this manner (Robb & Kestin 2002). The fish die in this way through anoxia, as the water around them drains off, resulting in the collapse of their gills. Kestin *et al.* (1991) showed that rainbow trout killed in this way took much longer to die as their temperature was lowered. Fish brought into air at 20˚C lost consciousness within 2.6 minutes, while at 2˚C this took 9.6 minutes on average while the fish showed full awareness and a degree of aversive activity before cold paralysis occurred in their muscles. Death by anoxia took longer at the lower temperature most likely due to reduced oxygen demand with the decreased muscle activity, while there was no evidence to show reduced awareness during this period. The conclusion is that this method is likely to be extremely stressful to the fish.

The requirement to chill the carcass for food safety and quality reasons has also led to the development of a method where fish are immersed in chilled water. This method is not designed to kill the fish, unlike the death on ice as mentioned above, but rather to cool them down rapidly. A benefit of this is that the rapid chilling also reduces fish activity after a period of time, so the fish can be handled and killed more easily.

Skjervold *et al.* (1999, 2001) developed this method for Atlantic salmon, showing clear benefits to carcass quality over carbon dioxide narcosis, which had been the preferred method for the industry, particularly in Norway. Commercially, fish (either Atlantic salmon or rainbow trout) are pumped from a well boat or from a holding cage into a tank containing chilled seawater. This water is run in a counter current to the incoming fish and then to a refrigeration unit, aiming to bring the water temperature down to between 2 and 4˚C. Commonly, due to the expense of chilling such a large volume of water, the water is reused for the whole day's operation, with some small top up as required. This leads to the deterioration of the water quality over the day, even if oxygen is added (Roth *et al.* 2006). The fish are moved through the tank by baffles, taking between 30 and 40 minutes as a general rule.

On entering the chilled tank of water, the fish commonly show one of two behaviours which depend on the temperature drop they experience (pers. obs.). If the temperature drop from ambient is relatively large (over 10°C seems a good guide from personal experience and discussions with operators), the fish show a large degree of swimming activity combined with attempts to escape. This will typically be intense for about 2 minutes and decrease over the next few minutes until the fish appear to be relatively quiet. On exiting the tank after 30 to 40 minutes, the fish appear to be muscularly exhausted, although if they are placed in fresh, well oxygenated water they will recover posture within about 5 to 10 minutes (Roth *et al.* 2006). This makes the bleeding process – carried out by cutting the gill arches with a sharp knife – relatively easy to carry out. In contrast, if the temperature drop is relatively small (less than 5°C appears to be a good guide), the fish are relatively quiet in the tank, but are very active when handled on exiting the tank. The bleeding process is then much more difficult as the fish react strongly to being held and gill cut.

If the process control is not good, too many fish may be pumped into the tank, resulting in the water heating up rather than cooling. This is particularly likely in the summer, when warmer ambient temperatures are encountered. Thus, the cooling effect on the flesh for food safety and quality reasons is lost, while the fish are still exposed to very poor water quality and stress. It is essential that the equipment is able to cope with the volume of warm fish entering the system and maintain the chilling effect.

In addition to the temperature insult, it is likely that the fish react to the poor water quality in the system. As the water is recirculated, it may receive some oxygen supplementation, but dissolved ammonia and carbon dioxide in the system remain and build up over the day. Mucus and any faeces or blood will also accumulate in the water. Fish will be able to detect these chemicals, coupled with any potential lack of oxygen, and will react strongly and aversively to them. It is therefore likely, though this is not confirmed, that fish reactions will increase over the day unless the water is freshened regularly.

Skjervold *et al.* (2001) showed that this method is stressful to the fish, with clearly elevated plasma cortisol compared to controls. However, the authors argued that the pre-handling was more stressful, over-riding any additional stress of rapid chilling, demonstrated by there being no significant difference between pre-handled control fish and live chilled fish in terms of plasma cortisol. However, as pre-handling procedures can be improved, as discussed above, so the impact of live chilling alone on welfare would increase.

In summary, this system does not stun the fish and there is no evidence of any anaesthesia. As fish are currently commercially taken from the live chilling tanks and bled directly, it must be concluded that this is carried out on fully conscious fish. If the ambient water temperature is greater than 10°C above the chilled water, the fish appear to be severely stressed by the temperature insult. If the temperature difference is less than 5°C there appears to be little effect of the temperature itself

on the fish. However, poor water quality in the system is also highly stressful to the fish, so the water must be regularly cleaned or changed. In practical terms, this is generally a system which stresses the fish, although the degree may alter, although the method appears to be less stressful than exposure to saturated carbon dioxide solutions. Improvements could be made commercially: minimum water quality standards should be set and maintained and fish should be stunned by another method on exiting the tank before being bled. However, the effect of the magnitude of the temperature insult on the fish would have to be investigated further.

Percussive stunning

A percussive stun results from the application of a high speed blow to the head, resulting in the brain shaking within the skull. This disrupts the neural processes there, causing a loss of awareness, which may be temporary or permanent. To achieve a good percussive stun, the object striking the fish head must be moving very rapidly as:

$$F = mv^2$$

where F is the force, m is the mass and v is the velocity of the object applying the force, so more force results if the object moves faster. The fish head must also be free to move after the blow, to achieve the shaking of the brain. If the head is clamped a stun will not be achieved (pers. obs.).

Percussive stunning has been used for a long time by anglers to kill individual fish, by using a wooden club (often termed a 'priest'). This was developed to a commercial stunning method for larger fish, such as Atlantic salmon. Typically, a lightweight club about 40–50 cm long made out of polypropylene or wood would be used. Fish would be pumped or netted onto a table, the operator would restrain the fish with one hand and hit it on the head with the club. The aim would be to hit the fish in the region shown in Plate 19, allowing the head to move down following the blow.

Operators would tire during this procedure, leading to weak or poorly aimed blows which did not stun the fish, but did stress them. Therefore, automated devices were developed. A range of such devices are currently available, each with different characteristics and ability to stun the fish. While some are very efficient at stunning the fish, others are not, with the mechanism moving too slowly to achieve an effective stun (pers. obs.). This means that each model requires assessment and approval with respect to its ability to stun fish humanely. No recommendations are made here with respect to this, but it is straightforward to assess the equipment effectively.

With the need to handle individual fish, percussive stunning was commercially viable on relatively large and relatively valuable species of fish (such as Atlantic salmon). Smaller, less individually valuable fish such as portion sized rainbow trout were not viable to stun in this manner. However, recent development of automatic

machines which will stun individual small fish without the need to handle them could change this.

Even with automation, percussive stunning has to be carried out on an individual basis. Due to this, a delivery system from the water to the point of stun has to be set up and this has the ability to impinge on the welfare of the fish. Often fish will be pumped or netted onto a table, where they will be out of water until they are stunned. If relatively few fish at a time are delivered to the table and the rate of stunning of the fish is high, the system will be fairly low stress. However, if too many fish are on the table or the stunning rate is low, fish will be out in air for several seconds and maybe as long as a couple of minutes before they are stunned (pers. obs.). Removal from water to air is a great stressor on the fish. Observations show that on exiting a pipe (where they are in water) and arrival on a table, the fish are relatively calm for up to 10 seconds and then become very active, flapping and obviously trying to escape. Beyond this time, activity is high, reflected by rapidly decreasing muscle pH until exhaustion sets in and the fish become still (pers. obs.). From a welfare point of view, it is obviously important to stun the fish and render them insensible before the onset of this stress.

To this end, a pump system which delivers a steady stream of fish, which can be stunned by operators around a table, is preferable to a batch system such as a brail. The pump should not deliver too many fish at a time though, so it is important that there is good communication between the stunners and the operators crowding and pumping the fish. To improve on this, an automatic system which keeps the fish in water almost up the point of stunning has also been developed. Fish are pumped into a header tank of water, from which they can escape down channels which lead into automated percussive stunners. By keeping the water quality high and ensuring that the fish move rapidly through the system, the aim is to ensure good welfare and a rapid killing rate.

Percussive stunning, applied correctly, provides an immediate loss of sensibility in rainbow trout, Atlantic salmon, and gilthead seabream, *Sparus aurata* (Kestin *et al.* 1995, Robb *et al.* 2000, van de Vis *et al.* 2003 respectively). Often with Atlantic salmon and rainbow trout, the blow will achieve an irrecoverable stun – in other words kill the fish. However, if the blow is too weak or misplaced, no stun is achieved. There can be a degree of species variation though. For instance, gilthead seabream must be hit on the side of the head to achieve a good stun; hitting them on the top of the head applies the force on to bone and muscle tissue which absorb the blow (van de Vis *et al.* 2003).

In summary, percussive stunning of certain species of fish, if carried out correctly achieves an instantaneous and often irrecoverable loss of sensibility – in other words the fish are stunned and may be killed. Manual application can be excellent with trained operators, but may result in poor stunning with fatigue setting in. A variety of automated systems are available, but should be assessed individually for efficacy. The delivery system to the percussive stunning area should be set up to minimise stress on the fish before they are stunned.

Electrical stunning

Electricity is used to stun a wide variety of food animals. A variety of applications have been developed for fish, but there is concern over the efficacy of some (Robb & Kestin 2002). Electricity operates on both nerves and muscles in the body (Lamarque 1990). An electrical stun will affect the operation of the nerves, preventing sensory nerves from passing information to the brain, or the brain from processing incoming information. However, electricity can also cause muscle paralysis, which may appear similar to the conditions resulting from an electrical stun while the brain is still fully functional. Electro-paralysis results from repeated stimulation of the muscle fibres, causing them to contract rapidly and so the animal stays effectively still. However, there is not necessarily any loss of sensation in this process and, as anyone who has received an electric shock will testify, it could be very painful and stressful.

Systems developed for fish range from in-water treatments to dry applications. At its most simple, fish can be netted into a tank and a current applied across the tank (Robb *et al.* 2002a, Morzel *et al.* 2003, Robb & Roth 2003, van de Vis *et al.* 2003, Lines & Kestin 2004, 2005). The current is left on for a period of time and the fish removed at the end. Some systems using this set up applied a very low voltage and current through the water for a long period of time (up to 10 or even 15 minutes). At the end of this time the fish would be very still, although later they could recover if transferred to fresh water. Such systems were almost certainly causing prolonged electro-paralysis to the point of muscular exhaustion, without any stun (Robb & Kestin 2002). If the system was run for a short period of time only – 1 second for instance – the fish would recover mobility immediately the current was switched off and would show a great deal of activity and escape behaviour, consistent with a very stressful experience (Robb *et al.* 2002a, b).

More recently some dry systems have been investigated. The fish are pumped up to a moving conveyor belt. On the belt they are taken through a curtain of electrodes which apply a stun to the fish (T. Benson, Humane Slaughter Association pers. com.).

Robb *et al.* (2002a) and Robb & Roth (2003) carried out a series of experiments into the electrical stunning of rainbow trout and Atlantic salmon, using both behavioural and VER methodologies. Using short duration applications of current, the effects of current, time and frequency were investigated. Stun duration – the time for which the animal remains insensible after the electricity is turned off – increases with increasing current and increasing application time, but decreases with increasing frequency over 50 Hz (no lower frequencies were investigated). Atlantic salmon and trout require relatively low current (Robb *et al.* 2002a, Robb & Roth 2003, Roth *et al.* 2003, Lines & Kestin 2004) compared to eels, *Anguilla anguilla*, and African catfish, *Clarias gariepinus*, for example (Robb *et al.* 2002b, Lambooij *et al.* 2004 respectively). If the aim of the electrical application is to kill the fish then current requirements vary even more (Robb *et al.* 2002a).

Orientation of the animal within the current appears to be important – whether the current flows across the head or along the whole body – although this may be a function of the voltage gradient across the animal (Lambooij *et al.* 2002a). There may also be differences in the current across different animals placed in the same tank (Lines & Kestin 2004), potentially making batch stunning an unpredictable operation.

Carcass quality was also affected by electrical stunning, with haemorrhages appearing in the flesh. This damage tended to occur more frequently with higher currents and lower electrical frequencies. Thus commercially, there needs to be a balance between the requirement to stun and the need to keep damage to a minimum (Roth *et al.* 2003, Poli *et al.* 2005).

To kill the fish with electrical current, the heart has to be stopped as well as the fish being stunned. However, the hearts of the fish species so far investigated seem to be highly robust, apparently defibrillating spontaneously after the electrical current is removed (Lambooij *et al.* 2002a). It would therefore seem to be necessary to either use a higher current to destroy the nerves controlling the heart, or to hold the fish in the current for longer so that the fish dies of anoxia before the heart defibrillates (Lines & Kestin 2005). For instance, Robb *et al.* (2002a) working on rainbow trout showed that with a 50 Hz current the fish could be killed during a short application, but with a higher frequency, a longer application was required to kill the fish. With eels, Robb *et al.* (2002b) and Lambooij *et al.* (2002a) used a higher current applied directly across the head to achieve a stun and kill at similar durations.

In summary, electrical applications can be used to stun and kill fish in a welfare friendly manner. However, there are a range of variables which need to be well controlled in order to achieve this. Each system needs to be carefully evaluated for efficacy, to ensure an effective stun is applied and that the animal remains stunned until dead, whether death is achieved by holding in an electrical current whilst maintaining the stun, or by some other method applied immediately afterwards, for instance bleed out or percussive stun/killing.

Other methods

A variety of other methods have been investigated for fish: for instance explosives, spiking and freezing. Robb & Kestin (2002) and Poli *et al.* (2005) recently reviewed the impact of these on both welfare and quality. As these methods are not generally used commercially, they will not be covered in this chapter.

Bleed out

Some species of fish are bled, as the presence of blood within the carcass will cause faster deterioration of the muscle after death and the blood also may cause unsightly marks in the flesh. Generally only the larger species are bled, such as salmon and

tuna, since it requires manual labour to carry out, which makes it uneconomical for small fish. Bleeding may also cause the death of the fish, in which case it is correctly termed slaughter (Anon 1995a). This may result following procedures which commercially do not cause a stun or kill the fish, such as live chilling or carbon dioxide narcosis. It also used to be carried out on fish directly from the cage, although this practice has mainly ceased now as it is much more difficult than bleeding a stunned fish.

Bleeding large salmonids is normally carried out by cutting the gill arches; the aim is to cut at least three of the four arches on one side of the head (Robb *et al.* 2003). With this number of major blood vessels cut, it takes about five minutes for an unstunned Atlantic salmon to lose brain function (Robb *et al.* 2000). During this time, the fish show a great deal of aversive reactions. This leads to the clear conclusion that bleeding unstunned fish is not acceptable from a welfare point of view. It also means that any stunning method applied to the salmon before bleeding must maintain the stun for at least this period of time to ensure the fish dies before it recovers from the stun. In practical terms, this basically means the fish should be permanently stunned by the method – or killed. From experience, it would seem that Atlantic salmon and rainbow trout which remain stunned for more than three minutes will not recover (Robb *et al.* 2000, Robb *et al.* 2002a). Applying this knowledge, there is no need for a requirement to bleed these fish immediately after stunning, because if they are not sufficiently stunned they will recover during bleed out even if this is carried out immediately. This contrasts with cattle and pigs, where the stun does not normally kill the animal, but to ensure no recovery, a minimum time from stunning to bleeding is set (Anon 1995a).

Conclusions

In summary of the harvest procedures with respect to welfare, it is clear that there are many different options used commercially. There are different welfare implications associated with the various options and within each one the application of best practice will help to reduce the impact. Fish take a long time to recover from a stressor and so it is important to address the welfare of the animals throughout the harvest chain, right to the point of death. The effects of any stressor will most likely be carried through to the end and the cumulative effect could be high. For example, Erikson *et al.* (1999) found that following the commercial pre-handling system which they investigated, bleeding of the live fish did not result in any extra stress. This clearly shows how poor commercial pre-handling systems can be.

Welfare throughout the harvest procedure is best addressed by raising awareness of the requirements. This has to be done at two levels commercially: with management to ensure acquisition of the appropriate equipment and with the operators to ensure that best practice is then applied. Training of both groups is essential in

order to achieve this. The link between good welfare and good quality (Robb & Kestin 2002, Poli *et al.* 2005) gives the commercial incentive for this to be applied in practice.

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Chapter 15

The Use of Stakeholder Focus Groups to Identify Indicators for the On-farm Assessment of Trout Welfare

Ben P. North, Tim Ellis, James Bron, Toby G. Knowles and James F. Turnbull

Introduction

In recent years, the welfare of all farm animals, including fish, has become an increasingly high profile issue and there is growing pressure on the regulatory authorities to legislate on fish welfare (FAWC 1996). Aspects of the feeding, husbandry, slaughter and the physical and social environments in which fish are farmed all have the potential to influence their welfare. Fish farmers are under increasing pressure from retailers and animal welfare organisations to address welfare issues in the commercial production cycle (Lymbery 2002). Consequently, there is a need to establish a framework that will ensure good fish welfare and provide the basis for a transparent and objective quality assurance system.

The welfare status of an animal can be described as lying somewhere on a continuum between good and poor welfare (Figure 15.1; Appleby & Hughes 1997), although this is undoubtedly an oversimplification of welfare, which is a multidimensional phenomenon. There are numerous examples of poor fish welfare, many of which were identified in the 126 recommendations made by the UK Farm Animal Welfare Council's Report on the Welfare of Farmed Fish (FAWC 1996). However, other than the absence of obvious signs of poor welfare, our understanding of exactly what constitutes good welfare for a farmed fish is very limited. In light of this, a pragmatic approach could be to focus on safeguarding fish welfare by identifying risk factors and indicators that are associated with an increased risk or prevalence of poor welfare.

Despite a recent upsurge in research into fish welfare there is no universally accepted method of welfare assessment, although it is widely acknowledged that no single indicator can adequately reflect the various aspects of welfare (Huntingford

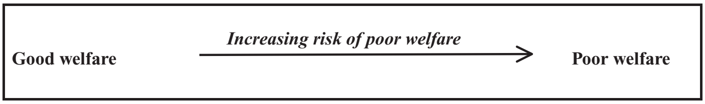

Figure 15.1 Schematic representation of the continuum between good and poor welfare.

et al. 2006). Previous studies have combined measurements of health, production, physiological and neurological responses in order to determine the welfare status of fish (Goede & Barton 1990, Turnbull *et al.* 2005, North *et al.* 2006, Varsamos *et al.* 2006). However, the relationship between stress, productivity and welfare is not necessarily easy to identify. Similarly, the extent to which short-term adaptive responses (physiological or behavioural) cause suffering is debatable, but prolonged, chronic stress generates a number of so-called tertiary responses (e.g. increased disease susceptibility, reduced growth and suppressed reproductive activity) that are established indicators of poor welfare (Huntingford *et al.*, 2006).

Dawkins (2004) proposed two questions to evaluate the welfare status of animals. Are they healthy? Do they have what they want? Recent innovative research in the study of terrestrial animal welfare has used behavioural preference testing to answer the latter of these two questions (e.g. Janczak *et al.* 2002, Dawkins *et al.* 2004, Taylor *et al.* 2006). The lack of vocalisations made by fish and the challenges associated with observing fish underwater mean that similar behavioural and motivational studies on a scale that would be meaningful for commercial aquaculture conditions are at present unrealistic. Aside from some very recent studies that have applied hydroacoustic technology to monitor swimming behaviour in caged Atlantic salmon (*Salmo salar*, Juell & Fosseidengen 2004, Johansson *et al.* 2006), there is a paucity of information regarding the behaviour of populations of farmed fish.

Most fish welfare research has been directed towards determining if the fish are healthy and growing well, with the assumption that good heath and growth is a meaningful indicator of good welfare (e.g. Turnbull *et al.* 2005). The success of such an approach is largely dependent on the quality of the indicators used to assess welfare. Furthermore, for welfare indicators to be useful for on-farm application, it is necessary that they are widely accepted and also practicable. Therefore, in addition to identifying objective welfare indicators through controlled studies it is important to consult stakeholders in the fish farming industry to establish their views with regard to fish welfare.

This chapter summarises the findings of a series of focus and discussion groups that were conducted with stakeholders in the UK trout farming industry as part of the Defra funded project investigating the interactions between water quality and welfare in farmed rainbow trout (*Oncorhynchus mykiss*). Focus groups are a form of group interviewing that incorporates the interaction within a group of individuals who are selected and assembled by researchers to discuss and comment on the subject of the research (see Powell & Single 1996, Morgan 1997). Focus groups are a recognised qualitative research tool and this form of stakeholder interaction

has been proposed as a valid and practical means on which to develop sustainable monitoring systems for farm animal welfare (Bracke *et al.* 2005).

The aims of the exercise detailed here were to explore the various criteria that could be used to evaluate fish welfare, and to identify stakeholders' concerns regarding the welfare of farmed trout. Stakeholders were drawn from fish farmers, fish veterinarians, animal welfare organisations, retailers, academics, and representatives from governmental and non-governmental organisations.

It was envisaged that by taking such an approach, a wide-ranging list of welfare indicators would be identified and they would be more universally acceptable. Although the main focus of this chapter relates specifically to indicators of trout welfare, in many respects the exercise was more far-reaching with both discussions and stakeholders' expertise extending into other areas of commercial aquaculture and other livestock industries.

This summary presents an interpretation of the main topics of discussion by participants in response to questions posed by the facilitators. The results are presented without ascribing the comments, suggestions or proposals to individuals. Areas of unanimous agreement or unresolved disagreements have been identified; all other comments originated from either individual participants, or a proportion of the participants. The views and comments summarised in this report are not necessarily the views of the authors, nor all of the respondents attending the focus groups.

Methodology

Stakeholders

Participants (attendees) were invited from various stakeholder groups in the UK trout farming industry, drawn from fish farmers, fish veterinarians, retailers, academics specialising in fish welfare, and representatives from governmental and non-governmental organisations (Table 15.1).

Process

Each focus group consisted of between eight and fifteen participants and lasted between 40 minutes and two hours. All focus groups were facilitated by Dr James

Table 15.1 Stakeholders, venues and dates of focus group meetings.

Stakeholders	Venue	Date
Trout farmers	British Trout Farming Conference, Sparsholt College, Winchester	September 2004
Fish veterinarians, academics, farmers, governmental and NGO representatives	Fish Veterinary Society Meeting, Edinburgh	November 2004
Retailers, governmental and NGO representatives	Novartis House, London	January 2005

Turnbull with the exception of four concurrent groups assembled at the Fish Veterinary Society (FVS) meeting. In order to maximise the usefulness of this exercise, the format of each focus group was tailored to fit the experience and expertise of each group of stakeholders. This approach was deemed to be more appropriate than adopting an identical process for all stakeholders, as the level of understanding of aquaculture varied considerably between the stakeholder groups. For example, farmers and fish veterinarians were already very aware of practical aspects of farming fish, whereas others were less so.

Analysis

All focus groups except for those carried out during the FVS meeting were recorded using a digital voice recorder (Sony, ICD-P28). Written notes were also taken and A1 flip pads were used for recording group responses to brainstorming and ranking exercises. The main points of each of the focus groups were transcribed and have been summarised into sections representing the main topics of discussion.

Farmers' focus group (Winchester, September 2004)

Process

The farmers' focus group took place during the 2004 British Trout Farming Conference at Sparsholt College (Winchester, Hampshire), with farm managers and stock workers invited to attend. After a brief introduction providing background information about the project, discussions were directed towards the subject of fish welfare and the group was introduced to the concept of assessing animal welfare by asking Dawkins's two questions (Dawkins 2004):

- Are the animals healthy?
- Do they have what they want?

The group was then asked to generate a list of indicators that could be used to answer these two questions. The focus group concluded with a more generalised discussion of other fish welfare issues; this was initiated by asking the group's views on a fish's capacity to suffer.

A total of 15 farmers representing eight different trout farming companies attended the farmers' focus group.

Results – Are they healthy?

Water quality

Providing fish with good water quality (WQ) was agreed to be the best way to ensure that they are healthy. Dissolved oxygen (DO) was mentioned at numerous

points during the meeting and was frequently identified as a major factor affecting fish welfare. One farmer stated that incidence of fin erosion, especially of the dorsal fin, was reduced dramatically if outlet DO was maintained above 85% saturation. Another said that he had seen improvements in quality, health and food conversion ratio (FCR) following the introduction of a liquid oxygen injection system. Other WQ parameters mentioned as important were ammonia and suspended solids. The participants agreed that if a farmer could provide records showing that key WQ parameters were maintained within specified limits, they would be demonstrating that they were safeguarding welfare. However, the practicalities of measuring water quality within every system on-farm were questioned.

Fin erosion

Fin erosion was discussed at several points and was generally viewed as a useful means of assessing fish health. It was considered to be important to differentiate between 'old' and 'new' damage, i.e. active damage (e.g. inflammation and bleeding), compared with historic damage (e.g. thickening or healed scar tissue). It was suggested that if severe fin damage occurred early in a fish's life history, the fins might not be able to fully regenerate. However, it was argued that a fish with healed stumps of fins could be healthy, in a similar way to a person with an amputated limb.

Some participants thought that the bacteria and parasite loads of farm water were a major factor influencing fin erosion, especially in the case of ectoparasites such as trichodinids (*Trichodina* spp.) and costia (*Ichthyobodo necatrix*). There was debate regarding the role of fin nipping in the development of fin erosion, with several farmers suggesting that damage resulting from aggressive interaction between fish would only occur in the case of prolonged periods of starvation. However, no consensus was reached on this topic.

The influence of stocking density (SD) on fin erosion was discussed, with one farmer suggesting that it was possible to produce 'fin perfect' rainbow trout fry at a SD of over 100 kg/m^3 provided that fish had enough oxygen and parasite loadings were low. The same farmer suggested that if outflow DO was never less than 7 mg/L it was possible to farm rainbow trout fry at SDs from 90–300 kg/m^3 until a size of 50 g without sustaining serious fin damage. However, a conflicting example was provided by another farmer of high incidences of fin damage occurring at low SD (10–15 kg/m^3) in large raceways with high rates of water exchange. There was a general agreement within the group that such conflicting experiences relating to the occurrence of fin erosion within the industry were commonplace.

Water chemistry was seen as a major factor influencing fin erosion, with pH considered to be of particular importance. Differences in water chemistry were thought to be a factor contributing to variation in observed levels of fin damage between batches of fish farmed under similar husbandry conditions on different farms. One farmer believed that water chemistry was the most important WQ parameter

affecting fin erosion and that fish were generally 'hardier' in acidic waters (pH < 7) due to lower numbers of parasites.

Several farmers viewed abrasion to be the initial cause of fin damage, followed by secondary infections. However, the role of abrasion was questioned with regard to dorsal fin erosion. The influence of different substrates on fin erosion was also discussed and the common assumption that concrete surfaces were more abrasive was questioned. Similarly, some participants questioned the value of husbandry innovations such as baffles and AquaMats®, which have been reported to reduce fin damage (e.g. Arndt *et al.* 2002).

The possibility of genetic and behavioural bases for susceptibility to fin damage were also discussed. It appeared to be generally accepted that within a given population of fish on a restocking farm there will be 20–30% of fish with no fin damage (referred to as 'firsts'), followed by around 40% that will have some damage ('seconds'), and a further 40% with more severe fin damage that would not be of sufficient quality for restocking and would be sold for table consumption instead.

Establishing exactly what constitutes an acceptable level of fin erosion and the actual implications of fin erosion for fish welfare were also discussed. It was proposed that a certain amount of damage was inevitable and that damage to fins occurred naturally in populations of wild fish. It was also argued that a fish could still achieve good growth with damaged fins. However, at least one farmer believed that it was rare to see a 'good fish' with bad fins. It appeared to be generally accepted that fin erosion could make fish more susceptible to diseases such as enteric red mouth disease (ERM, *Yersinia ruckeri*) and other secondary infections.

Behaviour

Although behaviour could be a potential indicator of fish health, it was agreed that it must be interpreted in the context of the 'normal' behaviour for a particular batch of farmed fish. It was acknowledged that quantifying behaviour would be very difficult although several examples of behaviour that farmers use to evaluate welfare problems were provided, such as reduced feeding behaviour, flashing (fish turning on their sides, thought to be associated with physical rubbing of the body against the floor and sides of rearing units), and fish colouration. The phenomenon of 'black fish' was mentioned: these were described as smaller fish (runts), dark in colour, that generally remained near the outflow of a raceway and were possibly subordinate fish.

Dominance hierarchies were mentioned several times and were viewed as being a major problem at lower stocking densities. There was no support for the hypothesis that fish swimming around in the same direction (especially in cage culture systems) represented stereotypic behaviour due to boredom, with a consensus that trout are shoaling animals that preferred to be in larger groups. Fish behaviour was viewed as differing between production systems, with other factors such as SD, water flow rates and WQ also having an influence.

Disease

Records of disease treatments were considered as a potential means of monitoring the health status of fish. However, it was argued that records of disease treatments could be a sign of both good and bad welfare, i.e. records of disease treatment could show that a farmer is doing everything possible to safeguard the welfare of his fish by monitoring health and treating when necessary, whereas a site that did not treat could be failing to observe or respond to health problems. It was seen to be important to separate prophylactic treatment from therapeutic treatment, although it was acknowledged that this would be difficult. The discussion moved on towards the problems associated with farms that were subject to high levels of mortality due to endemic recurring disease outbreaks, e.g. proliferate kidney disease (PKD) and ERM. It was suggested that the welfare of the fish on such farms might be unavoidably compromised.

Mortality

Mortality rates were suggested as presenting a useful indicator of the health of a stock of fish and by law, farmers are required to keep a daily log of mortalities. There was debate surrounding acceptable levels of mortality, especially in the case of farms situated in catchments with endemic disease problems. It was also emphasised that mortalities are naturally higher at some stages of production than others, e.g. hatchery versus grow-out, therefore the mortality rate would have to be related to the stage of the production cycle and to some extent the type of farming system.

Stocking density

It was acknowledged that there were welfare implications of excessive SD, but there was a consensus that the maximum SD should be considered individually on a farm-by-farm basis to take account for the different types of production system. Stocking density was not considered to be a useful indicator of welfare by the group, but there was agreement that if a farmer failed to remain within a specified range of key WQ parameters (e.g. DO) SD would need to be reduced.

Feeding

At several times during discussions the view came across that if a batch of fish is feeding and converting food well, it is an indication of good welfare. The discussion centred on the use of food conversion ratio (FCR, food fed : somatic growth) as a practical and meaningful means of assessing fish health. Although generally considered useful, it was argued that FCR would be limited to providing a historic perspective of welfare.

General comments regarding the selection of welfare indicators

It was agreed that welfare assessment needs to be considered in the context of the whole population, i.e. invariably when looking at a batch of fish it is the sick or dying fish that attract attention. It was emphasised that on a farm that may hold in excess of a million fish there will naturally be some sick or dying fish, in the same way that a similar sized human population will have sick and dying individuals, regardless of the resources available. The time scale of welfare assessment was suggested to have an influence on the choice of indicator, e.g. condition factor was suggested as a good indicator of long-term health. The appearance of gills (pale colouration as an indicator of poor health) and eyes (colour, cataracts, and damage) were also viewed to have potential as indicators of fish health.

Results – Do they have what they want?

Water quality

Good water quality was the first factor mentioned with regard to providing fish with what they might want. Several farmers felt this was a central issue with one stating: 'If you can demonstrate that you are providing fish with the environment that they love, you are providing the perfect conditions for welfare'. The WQ parameters considered the most important were DO, suspended solids, ammonia, adequate flow (water exchange rate and current), and water temperatures of less than 24°C. It was argued that some smaller farms would not be able to afford WQ monitoring equipment, although it was the view of at least one farmer that being a small farm should not justify incompetence and if a farm cannot afford a DO meter then they should not be allowed to farm fish.

Husbandry and duty of care

The maintenance of clean nets (for cage culture) and tank surfaces was considered important, as the accumulation of waste (e.g. uneaten food/faeces) could be detrimental to fish welfare. The importance of describing and enforcing husbandry protocols such as disinfection between batches of fish was also stressed, which led on to discussion of the concept of the 'duty of care', with reference made to the farm certification standards specified in the Quality Trout UK (QTUK) accreditation scheme. Providing adequate protection from predators and appropriate disease treatments were proposed as two means of demonstrating that the farmer was providing what a fish might want. Specific reference was made to the five freedoms of animal welfare (Anon 1992), with the general consensus that the five freedoms basically represent the things that any good farmer tries to do. There was the suggestion that safeguarding welfare was intrinsically linked to the economic viability of a farming operation, thus acting as a form of self-regulation, with one farmer

commenting: 'If the fish are not healthy they will either die or not convert food (well) and the farm will lose money'.

Feeding

Not feeding too much, or feeding too little food was considered to be important and reference was made to feeding protocols in the QTUK guide. Periods of starvation were also suggested to be important for fish welfare and there was consensus that any reference to periods of fasting should be in degree-days (i.e. based on water temperature) rather than specifying a conventional time.

Disturbance

The view was expressed that fish would probably want to be left alone. Knowing when to leave fish alone was seen to be important along with avoiding excessive disturbance and unnecessary handling. It was acknowledged that some handling is necessary and could ultimately benefit fish welfare, e.g. grading to prevent excessive size discrepancies and to regulate SD. Managing the frequency and timing of disturbances was suggested as being one way that farmers could improve fish welfare, e.g. avoiding handling if fish are already stressed.

Can fish suffer?

No consensus was formed within the group regarding a fish's capacity to suffer. Some farmers thought that fish were unable to suffer or experience pain in the same way as humans, while others believed they could feel pain despite the lack of any conclusive proof to support this. It was argued that if fish could not feel pain then farmed fish could be harvested in the same way as capture fisheries (e.g. suffocation on ice, or even live gutting). However, there was a general consensus that the industry could not afford to disagree with public opinion and should take a pragmatic view, regardless of personal opinions. Therefore, if it becomes widely accepted that fish do feel pain and there is a demand from the public for better welfare of farmed fish, farmers will have to accept this opinion and ensure that their products comply. One farmer pointed out that 10 or 15 years ago no one would ever have considered humane slaughter of fish, but now it is becoming common industry practice.

Other welfare concerns

Lack of licensed medicines

The lack of medicines to treat fish properly was seen to be a major fish welfare issue. Several examples were provided of treatments that had previously been effective

against common diseases (e.g. malachite green for treating fungal infections) but are now banned with no effective replacement.

Inspectors

Concern was expressed over the lack of education/understanding of the farming systems by government bodies and inspectors. There was deemed to be a lack of communication between government and farmers. Practical understanding of fish farming was seen to be a more important quality for inspectors to have than academic qualifications.

Factors outwith farmer's control

Deliberate sabotage and vandalism of sites was mentioned as being a potential welfare concern on fish farms. Pollution incidents, droughts and flooding were also mentioned. Although such occurrences were seen to be largely unpredictable, minimising the risk of such events was generally considered to be within the remit of a farmer's duty of care.

Transportation of fish

Fish transportation was seen to be a grey area that was currently lacking in regulation. Several farmers called for transportation to be more specifically regulated and saw a need for transporters to demonstrate that welfare was being safeguarded during transit. A system similar to that used for refrigerated shipping of foodstuffs was suggested, where WQ conditions for the period of transportation could be recorded and checked.

Production backlogs

Cancellation of orders for fish by processors/retailers was seen as a significant welfare issue as it potentially resulted in backlogs through the farming system. Examples were provided of welfare problems for harvest sized fish with no buyer, such as prolonged periods of fasting/restricted feeding resulting in loss of condition and high levels of mortality. However, there was a general acceptance that farmers had very limited power to change this situation, although one farmer suggested that it reflected a management problem if this was a regular occurrence. It was also reported that retailers/processors sometimes asked for extra fish, which encouraged farmers to pre-emptively 'push fish on' without a guaranteed sale. One hatchery owner pointed out that the situation was not restricted to harvest sized fish and that hatcheries experienced the same problem with cancelled orders for fry from on-growing farms. The question of how analogous situations are dealt with in other forms of livestock farming was raised. A proposed solution was to draw up a

contractual agreement of joint responsibility between farmers and retailers/processors (possibly brokered through the British Trout Association), although there was consensus that such action would only be possible if the whole industry formed a united negotiating block.

Summary of farmers' focus group

Much of the discussion centred on the potential for poor WQ to affect fish welfare with a consensus that DO was the single most important parameter, followed by suspended solids and ammonia. There was agreement that by providing good WQ, farmers could demonstrate that they were safeguarding fish welfare.

Fin erosion was discussed in depth and a consensus was reached that it is a useful indicator of welfare, although the need to distinguish between active and healed fin damage was seen to be important. There was concern regarding the use of fin damage as the principal indicator, since it was seen to represent just one aspect of fish welfare. A certain degree of fin erosion was seen to be both acceptable and inevitable in the aquaculture environment. Other suggested welfare indicators were behaviour (in the context of deviations from normal behaviour), mortality rates, feeding (activity, appetite and FCR), and the demonstration of a duty of care through good husbandry practice.

Although no consensus was reached regarding the capacity of fish to suffer, there was general agreement that if this became the accepted view of the consumer, the industry would need to take the same standpoint and implement appropriate measures, regardless of personal opinions.

There was disagreement regarding the value of records of disease treatments as a reflection of fish welfare, i.e. did a history of disease treatments indicate poor fish health, or was this a demonstration of a farmer fulfilling their duty of care? Transportation of fish was seen to be an area that is currently in need of more specific regulation. There was unanimous agreement that the lack of effective medicines and vaccines available to farmers was a major welfare issue.

Fish Veterinary Society meeting (Edinburgh, November 2004)

Process

The discussion groups took place on the afternoon of the final day of a two-day meeting focusing on fish welfare that was organised by the Fish Veterinary Society (FVS). Delegates at the meeting consisted of veterinarians specialising in fish health, academic researchers specialising in fish welfare, fish farmers, representatives from industry bodies (British Trout Association, Scottish Quality Salmon), UK governmental (Defra, Home Office, Environmental Agency) and non-governmental organisations (e.g. Royal Society for the Prevention of Cruelty to Animals, Compassion

in World Farming). Although some of the delegates were not working directly or exclusively with the trout farming industry, the meeting was a unique opportunity to discuss relevant issues with a wide range of well informed stakeholders in UK aquaculture.

In order to accommodate the large numbers, delegates were divided into four groups of between 12 and 14 people and discussions took place simultaneously. The primary objective of this exercise was to generate a list of potential on-farm welfare indicators and rank these based on their perceived levels of importance. Despite the introduction given to the focus groups, some respondents requested more information on the purpose of the exercise and how the results would be used. This information was provided with reference to the objectives in the original project proposal.

After approximately 40 minutes the facilitators for each group met and produced a summary of the four discussion groups, highlighting consistent themes and areas of agreement and disagreement between groups. This summary was then presented to all of the delegates who had reconvened into a single group and was open to further discussion.

Analysis

After the meeting, the contents of flip charts were transcribed and further detail was added by each of the facilitators relating to the topics discussed in their group. The exact format of each of the focus groups varied depending on the direction that the discussions took. The findings of the four focus groups have been combined and summarised under the key themes that existed between the groups.

Results

Context of welfare assessment

There was consensus across all the focus groups that the value of any proposed welfare indicator would vary depending on the context in which it was used, e.g. auditing of farms, quality assurance, inter-site comparison, regulatory inspection. The nature and immediacy of the response to the information collected was also viewed to influence the most appropriate choice of indicator, e.g. will the information be used to adjust the farm management on a day-to-day basis, or is it a record to demonstrate that the welfare of a population has been protected over an entire grow-out cycle. The wide range of affiliation of the participants allowed the discussion to extend beyond trout welfare and into other aquaculture species, most notably salmon.

Water and environmental quality

Water quality was ranked highly in all of the groups and was considered by most groups to be second only to behaviour in terms of its value as a welfare indicator.

One group proposed that WQ parameters could be separated into tiers according to their relative importance for fish welfare. Water flow and DO were considered most important, since fish can die quickly if either of these is too low. Other WQ parameters seen to be important were CO_2, pH, NH_3, water hardness and suspended solids, which one group concluded have a chronic rather than acute effect on fish welfare. Water temperature was also mentioned as being important as it influenced all of the other WQ parameters. Other water quality parameters might be considered to be of secondary importance.

Although there was universal agreement regarding the importance of WQ there was debate regarding how WQ monitoring could feasibly be incorporated into a system of welfare assessment. It was also suggested that time-series rather than single-point samples were required. Several farmers stressed that it was the changes in WQ parameters that generally caused problems rather than absolute levels. This point was illustrated with the following example: fish could have good welfare in water with 6 mg/L DO if they were acclimatised to it, but they might suffer if they were used to 10 mg/L which suddenly dropped to 6 mg/L. This was seen as analogous to altitude acclimation in humans.

Other environmental parameters discussed in less detail, but also considered to affect fish welfare, included structural fouling, pollutants, toxic algae and jellyfish (the latter from a personal experience of one salmon farmer).

Day-to-day welfare indicators

(1) *Behaviour*. Behaviour was ranked highly by all of the groups, but was acknowledged to be critically dependent on context of assessment and difficult to quantify. It was recognised that most behaviour occurs below the surface and may not be visible, although it was stressed that absolute levels of these behavioural measures were not necessarily the most important consideration. The possibility of using underwater cameras to monitor behaviour was proposed, especially in cage culture. The distribution (depth and spatial distribution) of fish was considered to be a useful behavioural indicator.

There was widespread agreement that the most useful observation would be deviation from 'normal' behaviour (e.g. swimming or feeding), which is a useful means of alerting farmers to potential welfare problems with examples including: (lack of) the response of the fish to the approaching farmer; clumping around inflows, gasping at the surface and increased ventilation rate as an indicator of stress or low oxygen; flashing or jumping as an indication of parasite infestation; and weak fish accumulating near the outflow of raceways.

(2) *Feeding*. Although feeding response could also be included under behaviour, it was afforded a separate heading as appetite and FCR were considered important by all groups. FCR and specific growth rate (SGR, % body weight gain per day) were widely viewed as sensitive indicators of welfare and something that all farmers scrutinise routinely.

(3) *Mortalities*. A change in daily mortality count was proposed as an excellent indicator of welfare problems. The phenomenon of 'black fish' was mentioned in one group as being important – referring to individuals that disappear from a system without being removed or accounted for as mortalities (N.B. this definition of black fish differed from the one given in the trout farmers' focus group). The number of missing fish can only be calculated at harvest, but the implications of these unaccounted fish could reflect things such as inadequate provision of protection from predators, inadequate recovery of mortalities, cannibalism, or inaccurate estimation of fish biomass at stocking and consequent over/underfeeding.

(4) *Research tools*. Oxygen consumption was proposed as a potential welfare indicator that could be measured as the change in DO from inflow to outflow of a tank or raceway. The measurement of cortisol concentrations from water in rearing units was also suggested as an indicator of stress, although this technique is still being developed. It was recognised that to be meaningful, both of these measurements would need to take account of flow rates, system volumes, and the biomass of fish. Although at present unlikely to be realistic in terms of day-to-day monitoring, they were considered to have potential use in the future or as research tools.

Targeted sampling of fish: autopsy-based examination

Autopsy-based sampling was proposed as a useful means of assessing fish welfare. Such sampling was considered to be expensive and some participants questioned whether its value would justify its cost, especially if used as part of routine sampling forming part of a farm's records. Although no consensus was reached, sampling of fish appeared to be considered to be more useful in the context of targeted sampling for a specific problem.

Several fish veterinarians stated that they already used the appearance of a variety of internal organs as macroscopic pathological indicators, e.g. gills (the consensus was that gills were most important), condition factor, liver (colour and presence of fatty deposits), kidney, spleen and gut.

Post-harvest measures

Feedback from fish processing factories was suggested to be an objective, quantifiable, and presently untapped resource. Deformities, fin damage, and scale loss were mentioned as useful post-harvest measures, although there was some discussion relating to the amount of damage that occurs during harvest and processing. The average and intra-batch variability for condition factor was seen to be of use in reflecting possible welfare problems. The same was also true for size distribution of a batch of fish, but opinions differed between groups with

regard to its usefulness as a welfare indicator since it would be affected by grading practices.

Other post-harvest indicators included cataracts, vaccination lesions, physical injury, and the presence of ectoparasites. Sexual maturation could also be assessed post-harvest, as maturing fish in production systems were viewed to be a welfare issue due to the loss of condition and increased disease susceptibility observed in sexually mature fish.

Farm records

All groups saw farm records as a useful and practical on-farm indicator, although it was acknowledged that they would only be useful in providing a historical perspective of fish welfare. Maintaining accurate and up to date records for each batch of fish was thought to be the best way to provide traceability and demonstrate good husbandry, e.g. for a site audit. Records of the following factors were suggested as having relevance to fish welfare:

- Mortalities – including the cause of death, e.g. deformities, predators, disease
- Water quality – DO, pH, suspended solids, flow rates/water exchange, temperature
- Production data – biomass, fish numbers, SD, growth, FCR, appetite
- Disease treatments
- Harvest data – method of slaughter, processor rejections (rate and reason)

Demonstration of good stockmanship

Suggestions grouped under the heading of stockmanship included a range of factors such as management and husbandry practices that could be a means of demonstrating that measures are being taken to safeguard fish welfare. It was proposed that farmers should be working to the same standard across the industry and several attendees said that there should be a recognised qualification for fish farming such as a General National Vocational Qualification (GNVQ), or some form of evidence of competence, such as a record of training and expertise.

Management practices such as minimising the amount of handling disturbance were advocated. The use of equipment such as fish pumps was viewed as a way to minimise stress suffered by fish during routine husbandry practices such as grading or moving. The general condition of a farm was also seen to have potential implications for fish welfare, with factors such as the provision of adequate predator protection, quality of site equipment (e.g. pumps and graders) and nets (condition and fouling) all highlighted as important considerations.

The presence of a veterinary health plan, or health and welfare strategy was suggested as another means of demonstrating good stockmanship. It was proposed

that such systems should be based on risk assessments following the same format as hazard analysis critical control points (HACCP), or hazard analysis and risk assessments (HARA) used in factories and laboratories. Finally, education and communication was perceived as being essential at all levels (within site, between companies, and with legislators).

Summary of FVS meeting

Although the format of the four concurrent focus groups varied, there was generally a high level of agreement with regard to both the lists of welfare indicators and their relative levels of importance. Fish behaviour was ranked highly in all groups although it was acknowledged that it would be difficult to observe or quantify in the commercial aquaculture environment. This suggests that although behaviour serves as a very good day-to-day indicator for alerting farmers to problems, it will be of less use for audit/regulatory purposes.

Water quality was also ranked highly in all groups with DO again considered the most important parameter. The time scale of WQ monitoring was considered to be important, with time-series WQ measurements recorded as part of farm records seen to have potential for demonstrating that welfare had been safeguarded.

Maintaining accurate and up to date farm records was viewed to have potential for providing transparency and traceability, especially for external audits. Thorough and accurate record keeping would also provide a means of demonstrating good stockmanship. Details could be maintained for each batch of fish and provide information regarding mortality rates, growth, SD, feed consumption, FCR and WQ data (most importantly DO) and origin.

Harvest-based measures were considered to represent an objective and quantifiable means of assessing fish welfare. Tracing specific batches of fish back to the farm of origin is achievable as the number of rejected fish in a given batch of fish is already routinely recorded because it affects the price a farmer receives for his fish. Numerous post-harvest measurements were considered to have potential as welfare indicators, although it was recognised that it could be difficult to differentiate between injuries that occurred earlier in the farming system and damage that occurred during harvest.

While there was generally a high level of agreement between groups, there were some areas of dispute such as the implications of variability of size and condition factor within a batch of fish and whether this implied good or bad welfare. There was also some dispute regarding automation on fish farms, with the suggestion that farmers could become removed from contact with their stock if levels of automation increased.

The selection of the most appropriate indicators of welfare was recognised to be dependent on the context and purpose of sampling. Concern was expressed that

any system should not just be a checklist and that interpretation should be context and site specific.

Retailers, NGOs and Governmental organisations' focus group (London, January 2005)

Process

The final focus group took place at Novartis House, London and involved representatives from supermarket retailers (Marks & Spencer, Somerfield, Tesco), animal welfare orientated NGOs (Freedom Food, Humane Slaughter Association, Royal Society for the Prevention of Cruelty to Animals, Universities Federation for Animal Welfare, World Farm Watch, World Society for Animal Welfare) and governmental organisations associated with the UK aquaculture industry (Aquaculture Wales, Centre for Environment, Fisheries and Aquaculture Science Fish Health Inspectorate, Fisheries Research Services). All of the major supermarket retailers in the UK were approached (Asda, Co-op, Marks & Spencer, Morrisons/Safeway, Sainsbury's, Somerfield, Tesco, Waitrose), although only three were able or willing to send representatives.

The format for the focus group was less structured than previous groups and for the most part it took the form of an informal discussion centred on establishing the views and priorities of the respondents relating to fish welfare issues, implementation of on-farm welfare monitoring systems, and establishing acceptable/unacceptable levels of welfare.

Analysis

The dialogue was recorded and the main discussion points were transcribed along with notes taken during the discussions. The following subheadings reflect the main topics of discussion.

Results

Welfare priorities

The need for useful welfare indicators calibrated by research was seen as a priority, as was the need for research to establish which aspects of farming adversely affect specific aspects of fish welfare (e.g. behaviour, fin/tail injuries, and performance), along with thresholds for defining acceptable and unacceptable welfare.

The danger of using inappropriate selection criteria for the domestication of fish was suggested as a welfare priority; battery hens were used as an example of how inappropriate selection criteria can result in poor welfare. Stocking density and

slaughter methods were also cited as welfare priorities and live chilling of fish was specifically identified as being inhumane.

On-farm welfare assessment

There was consensus that any form of welfare assessment would need to be calibrated by scientific research. It was also suggested that welfare indicators would need to be practical things that could be observed and measured. Levels of injuries and deformities were seen to be useful indicators, although the severity of any abnormality would also need to be considered. Water quality was suggested to be important although the practicalities of measuring aspects of WQ on-farm were questioned. There was debate regarding the value of production-based parameters as welfare indicators, since good productivity was seen to be achievable even when welfare is compromised; the rapid growth achieved by broilers with leg deformities was cited as an example.

Harvest-based measures were suggested to have the potential to provide a historical indication of the level of welfare of a batch of fish. Reference was made to a system used in the Danish poultry farming industry in which a defined number of birds from each batch are inspected and scored at the processing plant; if the score is poor, then actions such as forced reduction in stocking densities can be imposed on the farm. Post-harvest inspection was also suggested to have potential to reflect the welfare of fish during harvest; the time taken until the onset of rigor mortis was cited as a useful indicator for terrestrial livestock.

Any workable system was seen to ultimately be restricted to observations of the physical appearance of the animal, the presence or absence of pathogens or parasites, and measurements of some aspects of WQ. The question of how well any such system of assessment would reflect the welfare of the animal was also raised.

Acceptable standards of welfare

It was agreed that the following factors needed to be taken into account when considering acceptable levels of fish welfare: severity of suffering (mild versus severe), duration of suffering (short versus prolonged), and the numbers of animals affected.

There were differences in opinion regarding where to 'set the mark' in terms of specifying acceptable standards of welfare. It was the opinion of some participants that it is best to focus on realistically achievable aims. However, a representative from a welfare accreditation scheme argued that the aim should be for the 'ideal', and using the example of fin erosion, suggested that any proposed system should aim for a 'fin perfect' fish. They went on to suggest that although the 'ideal' may be unattainable in the short-term, it was the best way to bring about long-term improvements. If a farm failed to achieve the standard, but could demonstrate that it was doing everything possible within the constraints of the industry and current

levels of understanding/available technology, then this would be acceptable. The discussion relating to acceptable levels of fin erosion concluded with agreement that improved understanding of the causes and control of fin damage should be a research priority.

Branding and communication

It was suggested that there is not necessarily a conflict between the cost of production and good welfare since good welfare can result in improved productivity. However, one of the retailers believed that there was generally a trade-off between the cost of production and welfare, which was influenced by the market's perception of a particular system of production and the relative value that customers attribute to it. The same retailer representative viewed communication of any welfare standards as being essential, since implementing welfare schemes was considered to come at a cost that has to be recouped from the customer. They went on to say that if the customers were unaware of the measures taken to safeguard welfare, they were unlikely to attribute a value to these measures and could be drawn to cheaper products produced without welfare guarantees.

Consumer perceptions of welfare

One of the retailers suggested that consumer perception of fish welfare might vary depending on the type of fish product they were purchasing. Based on experience from terrestrial meat marketing and using salmon as an example, they proposed that the level of concern regarding the fish's welfare would be greater if a consumer was purchasing a fillet, or whole fish compared with a sandwich containing salmon. Another example from research into meat marketing was also cited where customers who purchased products from the in-store meat counter were shown to have a greater level of concern regarding the welfare of the animals compared with customers buying pre-packed meat products from the shelf. It was agreed that an investigation into the consumer perception of fish welfare was outwith the scope of the current Defra research project, but key-informant interviews with consumer group representatives was suggested to be of potential use.

None of the participants were aware of any research that had been conducted into consumer perceptions of fish welfare and it was agreed that animal welfare NGOs, rather than consumers themselves, were the driving force behind the increasingly high profile of fish welfare.

System and species specific considerations

There was deemed to be a need to consider individual characteristics of each farming system, especially in the case of SD, with some systems suggested to be capable of supporting higher stocking densities than others. Species specific considerations

were also seen to be important and the relative tolerance of Pacific and Atlantic salmon during well boat transfer was cited as an example.

Summary of the retailer, NGO and governmental representative focus group

Some of the respondents had considerable experience with welfare monitoring and control in terrestrial livestock production and although they may not have been familiar with all of the major welfare issues relating to farmed fish, the focus of the discussion moved quickly towards the practicalities of implementing systems of welfare monitoring and control.

Harvest-based measures were again suggested as being able to provide a practical and useful indication of both the welfare during production and the welfare during slaughter. There was a consensus that operational welfare indicators should be practical and measurable although there were differences of opinion regarding the setting of target levels for welfare, with some respondents suggesting that the aim should be realistically achievable, while others suggested it was better to aim for the 'ideal'. The importance of the severity, duration and number of animals affected were particularly relevant points that should be taken into account when considering acceptable levels of welfare.

It was apparent that there was much that could be learnt from welfare standards and systems that have been introduced for other forms of livestock production with broiler and battery hens providing numerous examples. There was no knowledge of any research that had been conducted into consumer perceptions of fish welfare, although previous studies examining consumer perceptions of meat products were suggested to be of some relevance. Communicating any welfare standards to the consumers was expressed as being necessary to recoup the cost of implementation.

General discussion of authors

There was generally a very high level of agreement between the various focus groups regarding the most important factors related to assessing fish welfare. It was recognised that the selection of the most appropriate indicators will be largely dependent on the context and purpose of the assessment. The main generic areas of welfare indicators and their potential uses/outputs are presented in Figure 15.2.

Operational welfare indicators (OWIs)

Behaviour was considered to be a very important OWI by all stakeholders, although there was universal recognition that there would only be a limited ability to quantify behaviour on commercial fish farms; even if behavioural observations were limited to changes from the norm, this was still considered to represent the best early

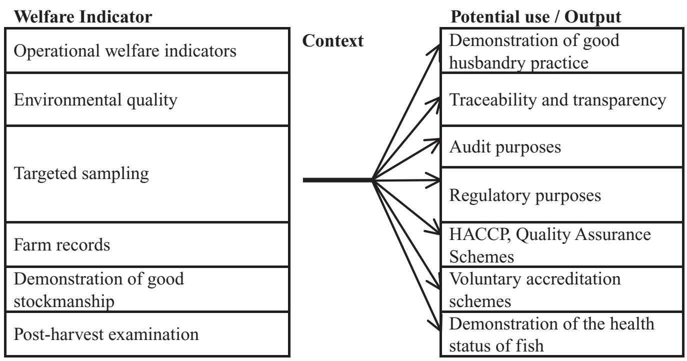

Figure 15.2 The main welfare indicators and potential uses for a system of on-farm welfare assessment.

indicator of potential welfare problems for a farmer. Feeding behaviour (appetite) and FCR were also considered to be useful OWIs on the premise that good appetite and FCR would be indicative of good welfare; most farmers already pay a great deal of attention to both these parameters and the necessary data would be readily available. Other frequently mentioned OWIs included mortality rates and condition factor. Fin erosion was generally accepted to be a useful OWI and the causes and implications of damaged fins were discussed in depth by all focus groups. There was debate regarding acceptable levels of fin erosion and also concern that any system of welfare assessment should not be overly dependent on assessing fin damage as it represented just one aspect of welfare. It was seen to be important that any form of assessment of fin damage should be able to separate active damage from historical damage.

Environmental quality

Water quality was considered to be the most important aspect of the fish's environment, with DO being the single most important parameter. Other important WQ parameters included suspended solids, ammonia and carbon dioxide. The complexity of interactions between different parameters, temperature and the chemical characteristics of a water source were also recognised to be important considerations. There was, however, some debate regarding the feasibility of measuring WQ on-farm. Other suggested indicators of environmental quality included availability of water, pollutants, structural fouling of nets, quality of on-site equipment and toxic algae blooms.

Farm records

The maintenance of accurate and up to date farm records allowing distinct batches of fish to be tracked through the production system was widely viewed to represent

a transparent, practical and meaningful indication of the welfare of a group of fish. There was a general consensus that recording the following information could provide an indication of the welfare of a particular batch of fish: mortality (including the cause of death); WQ (especially DO, flow rates, pH and water temperature); production data (e.g. biomass, SD, growth, and appetite); and disease treatments. It was apparent that most farmers already record some or all of this information. It was recognised that a limitation of farm records would be that they could only provide a historical perspective of fish welfare.

Sampling of fish

The following parameters were considered to be indicators that could be used in post-mortem-based assessment of fish welfare: condition factor; fin measurements; and inspection of the gills, liver, spleen, gut and kidney. However, there were reservations regarding the cost efficiency of such sampling on a routine basis, with consensus that it should be used in a targeted manner to investigate specific problems.

Demonstration of good stockmanship

Discussion points grouped under this heading encompassed the different ways by which farmers could demonstrate good stockmanship and satisfy their duty of care to their fish. It was suggested that some form of pan-industry qualification or award of competence based on records of training should be implemented. The general condition of the site was also considered to have potential implications for fish welfare including the provision of adequate predator protection, and the quality and cleanliness of on-site equipment (pumps, graders, WQ monitoring equipment, nets). The presence of protocols for husbandry procedures (e.g. biosecurity and disinfection, disease treatments) and well maintained farm records were other factors that could demonstrate good stockmanship. Many of the means of demonstrating good stockmanship were thought to already be in place on most well managed fish farms.

Post-harvest measures

Feedback from fish processing factories was seen as an objective, quantifiable, and at present, untapped resource. Deformities, fin damage, condition factor and scale loss were cited as important factors reflecting the welfare of a batch of fish. Other post-harvest measures included cataracts, vaccination lesions, physical injury, and parasites. The difficulty of separating damage occurring during harvest/processing from injuries that occurred during the production cycle was seen as a limitation of post-harvest monitoring. Parallels were drawn with existing schemes in the poultry industry for inspections of leg/feet deformities.

Potential outputs/uses for welfare assessment

Possible purposes of welfare assessment would include audit or regulatory purposes, forming the framework for HACCP/HARA systems, and for quality assurance and accreditation schemes. The potential benefits would be improved traceability and transparency through the farming system and would provide farmers with a means to demonstrate fulfilment of their duty of care.

Other points of discussion

The transportation of fish was seen to be an area in need of more specific regulation. It was suggested that a record of key WQ parameters should be provided for the duration of transportation. At the time of the focus group, fish were afforded protection under the Welfare of Animals (Transport) Order 1997, which requires that fish must not be transported in a way that causes, or may cause unnecessary suffering. In 2006, this Order was revised to provide further provisions, although this was initially only enforced in England. The order specifies that transporters should carry fish in suitable containers or means of transport with regard to the space, ventilation, temperature and security and with a supply of liquid and oxygen appropriate for the species. However, it was apparent that some respondents did not consider this to be sufficient in practice.

The welfare implications of the size variation within a given population of fish is an area that may warrant further research as there were conflicting opinions regarding the connotations for fish welfare. The lack of effective licensed medicines was widely recognised to be making it increasingly difficult for farmers to treat disease problems. The apparent lack of effective replacements for banned treatments that were previously used against common problems was seen to represent a major threat to fish welfare.

It was considered important to inform consumers of any measures that are introduced to safeguard or promote fish welfare – otherwise it would be difficult for consumers to differentiate between products produced under different levels of welfare protection.

Opinions of consumers

Within the remit and constraints of the Defra funded project of which this exercise was part of, it was only possible to carry out a pilot focus group with consumers. However, contact was made by telephone with several key informants from organisations representing consumers. A senior representative from Which? (formerly the Consumer's Association) was unaware of any work that had been carried out to investigate consumer perceptions of fish welfare. Similarly, a representative from Sustain (the alliance for better food and farming) stated: 'fish welfare is currently below the radar of consumer perception'. However, it was suggested that

consumer interest in fish welfare would increase in the event of sufficient media attention, especially in the case of a major food scare. An example of the potential for such incidents to affect consumer behaviour can be found in a report for the National Consumer Council, where organic salmon producers in Scotland were cited as reporting a 10% increase in sales in the week following the publication of a report linking farmed fish to an increased risk of cancer (Bush 2004). The only other literature we were able to find relating to consumer perceptions of trout was restricted to the marketing of trout (Shaw & Gabbott 1992) and identifying factors associated with consumer preferences for trout as a food product (Foltz *et al.* 1999). Considering the increasing profile of fish welfare and the level of investment in research, it would be timely to conduct a study of consumer opinions of fish welfare.

All stakeholder groups involved in this study agreed that the UK trout industry deserved credit for its proactive stance with regard to fish welfare.

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Part III
Ornamental Fish and Welfare

Chapter 16
The Welfare of Ornamental Fish

Chris Walster

Introduction

Ornamental fish have been kept for at least 1000 years, both for their beauty and for cultural reasons (Fossa 2004). Many species are bred in captivity, particularly the majority of freshwater species by numbers kept, and several marine species, and these species can therefore be considered to be domesticated. Yet any discussion of ornamental fish welfare is bound to produce heated debate, and any statements made are as likely to be agreed with as disagreed with by the reader. Since any fish has the potential to be kept as an ornamental fish, and the variety of systems used to keep them ranges from a humble goldfish set up to a large public aquarium or lake, it is probable that to discuss their welfare fully would require the writing of a textbook. However, if one were to restrict oneself to facts that are not disputed then one would still end up with a textbook on aquarium or pond management.

There has been little scientific research into ornamental fish and an attempt to produce a peer reviewed scientific journal a few years ago was abandoned due to a lack of suitable papers. That said, it is clear that scientific, government and public interest in ornamental fish is increasing and it is an area that requires further research. This is perhaps indicated by the fact that the European Union (EU) had included a suitable definition of what constitutes an ornamental fish in the draft proposals (Torgersen & Hoffman 2004: SANCO/10498/2004 rev 3b) for the council directive to replace EU Directive 91/67, although the latest draft appears to have shortened this to the first 20 words (Torgersen & Hoffman 2005: SANCO/14117/2/05 rev 2):

'Ornamental aquatic animals means aquatic animals which are kept, reared or placed on the market with an ornamental purpose only, and which are held in aquariums or ponds, public or private, that are either closed systems without direct contact to natural waters in the Community, or equipped with effluent disinfection systems'. The use of the phrase 'ornamental aquatic animal' recognises the fact that various invertebrates such as corals and shrimp are also kept within ornamental fish aquaria.

The Companion Animal Welfare Council (CAWC) recognises ornamental fish as companion animals, which means that society expects owners and producers to provide suitable care, but equally recognises the benefits that the keeping of companion animals brings to society. In the CAWC Report on the Welfare of

Non-domesticated Animals kept for Companionship, the definition of companion animal used was: 'all animals kept by private individuals or private groups for companionship, interest or hobby, together with those kept for related purposes such as breeding, supply, or education' (CAWC 2003). This definition of companion animals is useful as it provides the reasons why people keep ornamental fish.

The aim of this chapter is to indicate some of the current concerns regarding welfare both at the production stage and within the hobby, illustrate the diversity of opinion, allow the reader to form an assessment of the current status of welfare within the industry and hobby and suggest some possible ways forward.

Trade statistics

The worldwide trade in ornamental fish is estimated to be worth around US$5–6 billion according to the Ornamental Aquatic Trade Association (OATA). In the UK, the industry is estimated to be worth £300 million per year, the bulk of which is sales of equipment and food. The import value of fish is approximately £14 million. There are no exact figures of the number of hobbyists involved but it is thought that over 14% of households in the UK keep fish. This gives in excess of 3 million ponds and aquaria in the UK containing some 143 million fish.

Over 1000 species of freshwater and 1000 species of marine organisms are imported into the UK, of which the vast majority enter through Heathrow airport. It is estimated that approximately 36–38 million fish a year pass through the Animal Reception Centre at Heathrow. Other sources of supply include coldwater species farmed in the UK, tropical and marine species reared commercially within the UK or supplied through hobbyist networks and the capture of wild British indigenous species.

Production

The supply of ornamental fish to the trade is either through farming or wild collection. Farming ranges from small-scale family run farms, particularly in the Far East and the cottage industry of tropical fish production in the Czech Republic where part of the household, say one room, is set aside for commercial fish production; to large commercial farms, particularly those providing higher value species, although there are also large farms in Venezuela producing guppies (*Poecilia reticulata*) and various monoculture guppy farms in Singapore that have been established for many years. Within the UK, several types of coldwater fish belonging to the carp family, several species of freshwater tropical species and some tropical marine species are produced commercially. There is also a small market in hobbyist-reared fish. Indeed, it is often considered by the hobbyist that the fact fish have bred indicates good management of the stock and good welfare.

Wild capture mainly concerns marine species, particularly those associated with coral reefs and freshwater species from South America, in particular Amazonia, and to a lesser extent Africa and Asia. Much of the wild capture is carried out by indigenous peoples as a source of additional income and may be the only cash income in some cases. The fish captured are then transported to commercial concerns from which they are exported around the world.

Each method of production raises a variety of welfare concerns varying from health issues such as the levels of fish mycobacteriosis in farmed fish (notably *Mycobacterium marinum*), to the method of collection employed for wild fish. Health concerns will be discussed separately but there are four major welfare concerns, and one minor, which are specific to ornamental fish production.

Culling

Ornamental fish need to be of a suitable quality to sell. This means they should have the 'desired' body shape and colouration (note that many domesticated ornamental fish are selectively bred for features that deviate from the wild type, as is indeed the case for most, and probably all, other domesticated animals). For farmed fish, the quality can be controlled through selective breeding programmes. However, in species that do not breed true, then regardless of how selective the breeding programme, a large percentage of the offspring will be culled. This is particularly of concern with koi (*Cyprinus carpio*) where only a few percent of the offspring will have the correct colouration. Indeed, culling rates of over 90% can occur. This dramatic figure is somewhat ameliorated by the fact that under natural conditions it is likely that only a few percent of offspring will reach maturity. Fish farmers are thus applying a similar level of selective pressure, albeit in a different direction to that which occurs naturally. It is worth noting that the environment in which these selected varieties are kept is far removed from that experienced in nature by their wild brethren. It is also known that some farms in Japan continue to raise these fish until they are of a suitable size to process for fishmeal. From a welfare perspective this still leaves the question: is it right to spawn fish knowing that a large number of the offspring will be killed due to human whim and what is our responsibility to them?

Collection from the wild

Wild collection probably evokes the most controversy in the production of ornamental fish and probably is the most complex issue regarding production since it involves more than just ornamental fish welfare, impinging on environmental and human welfare issues. The industry and the hobby in the UK would not condone indiscriminate and inappropriate capture methods. Indeed, standards setting bodies such as the Marine Aquarium Council (MAC) provide guidelines which are audited to ensure compliance by certified companies. Methods of capture such as

the use of sodium cyanide are condemned by all of the trade bodies both abroad: such as Ornamental Fish International (OFI), and the Pet Industry Joint Aquarium Council (PIJAC) in the USA; and in the UK, the Ornamental Aquatic Trade Association (OATA). Other unwelcome capture practices include physical invasive capture methods, such as the use of a two-pronged stabbing pole to spear the mandarin fish (dragonet, *Synchiropus splendidus*) in the Philippines (Sadovy *et al*. 2001). Over-fishing for the ornamental trade is reported to have occurred in many areas of Asia and South America. This is reported to have led to steep declines in stocks and to the local demise of certain species of ornamental value: as is well documented for some Asian freshwater fish, such as the harlequin rasbora (*Trigonostigma heteromorpha*) and the bala shark (*Balantiocheilos melanopterus*) (Lim & Ng 1990, Ng & Tan 1997). These declines led to a concomitant increase in prices, with the increased price giving the incentive for the species to be farmed. An alternative opinion is that the trade has not caused these declines and that they are due to environmental degradation caused by development projects and other causes (Kottelat & Whitten 1996). More recently, as concerns over welfare have increased and the ability to breed species in captivity has improved, companies such as the Tropical Marine Centre in the UK have invested in facilities and research to breed certain marine species in captivity. It is worth mentioning that historically, concern has focused on the marine ornamental fishery, but more recently conservationists and fish ecologists are also concerned about the sustainability of many freshwater ornamental fisheries. Interestingly in some species, such as freshwater angelfish (*Pterophyllum scalare*), wild stocks can command higher prices than farmed fish.

Is wild capture detrimental to welfare? When CAWC considered the wild capture of non-domesticated species it made the following points:

> 'CAWC robustly supports the enforcement of conservation legislation aimed at the prevention of over-exploitation of wild populations. Where wild populations are thriving, harvesting, providing it is regulated and sustainable, does not (by definition) present a threat to population viability. But does this practice represent a threat to welfare? It can do, but does not necessarily do so. Where standards of husbandry are high, welfare can be better in captivity than in the wild. Life in the wild is hazardous, needs are not always met, and in the context of the survival of the fittest, the less fit frequently face food shortage, injury, disease and lingering deaths. Accordingly, there is no compelling argument on grounds of welfare that animals should never be taken from the wild.'
>
> – CAWC 2003

On an individual fish basis, welfare is possibly decreased even when capture and transportation are optimum. However, when considering a batch of fish, then welfare is possibly not degraded, since a large number of those fish are likely to suffer predation and other causes of death naturally in the wild, even though there is a greater likelihood of death from prolonged stress after capture. The argument is

obviously which is better: a potentially swift demise when predated naturally, or the potential of death from prolonged stress? If one looks at the welfare of the species, then wild capture is not detrimental so long as the rate of capture is sustainable and it may even improve welfare. To draw the final conclusion the following points need to be considered:

- Marine fish are often captured when young and at a point when the natural instantaneous rate of mortality is high. Thus some captured fish would not have survived to maturity.
- Since a requirement for the ornamental trade is that the fish be a suitable size then mature specimens are often unsuitable.
- An ornamental specimen is worth a lot more (up to one or two orders of magnitude, particularly with reference to some of the more desirable South American catfishes) than it would be for food. Marine fish and corals sold internationally are usually 100 times the going rate of the same material sold locally for either food or building material
- Given an increased income there is less pressure for fishing, plus an incentive to maintain the source of income, but this ideal can be negated by human greed. Equally, if this livelihood option using a very small amount of biodiversity is denied, local fisherfolk then often ascribe much lower values to the habitat and require greater amounts of biomass to be exploited for the same financial return.

Therefore, taking ornamental fish from the wild can lead to less environmental degradation and the presence of more mature specimens for breeding.

The point is perhaps best illustrated by Project Piaba. This project, which has run for over 10 years, is based on the Rio Negro, a tributary of the Amazon. The main species (by number) captured here is the cardinal tetra (*Paracheirodon axelrodi*). The project carries out much, varied scientific research, but also educates the fisherfolk on appropriate methods of catching, improving holding facilities and how best to forward the fish on to Manaus. The project has seen dramatic decreases in losses sustained due to incorrect transportation to Manaus; shown that the fishery is sustainable; improved the income of the native peoples; and taught them that they need to protect the environment. The slogan of Project Piaba is: ‘Buy a fish, save a tree’ which is very apt (Chao*et al.* 2001). It would seem clear that where a fishery is properly managed then there is no detriment to welfare.

Cosmetic manipulation

In recent years, some ornamental fish species such as glassfish (*Chanda ranga*) and Corydoras catfishes (*Corydoras* spp.) have been injected with various coloured dyes to ‘improve’ their commercial value. Injecting glassfish has been condemned by trade bodies and also by hobbyists. It has also been shown that glassfish that have been injected with these dyes show increased infection with Lymphocystis virus which produces a cellular hypertrophy causing raised wart-like lesions on the

skin of the fish (P. Burgess pers. com.). These practices, which confer no benefit to the fish, should be condemned on welfare grounds. OATA has recommended that its members do not trade in dyed fish of any type.

Genetic modification

Genetically modified (GM) species have been produced in the Far East, notably in Singapore, and have been sold in some states of America. Under current legislation (Directive 2001/18/EC), no ornamental GM fish have been imported into the UK and this legislation requires extensive risk assessments to be carried out before this could be done. OATA has already stated that the introduction of GM technology is an unwelcome addition to the trade.

Withholding food

For coldwater fish and others which are sold on size, there is a slight issue over how producers maintain batches of fish at the correct market size. This is achieved by slowing growth rates by decreasing food availability. Although this is an accepted management practice it can lead to 'weaker' fish and this is manifested in decreased health. This is particularly relevant in smaller sizes of koi (*Cyprinus carpio*). However, if this management technique is applied correctly it need not result in compromised welfare and is a technique widely used in the management of many other farmed species.

Transportation

The transportation of fish is, just like other animals, covered by extensive national, regional and international laws and codes of practice (see Chapters 6 and 11). There are few, if any, scientific papers regarding the level of mortality of ornamental fish during importation to the UK, but Huntingford *et al.* (2006) cite two papers and conclude that mortalities can be as high as 30% during capture, a further 5–10% during transport, and as high as 30% during acclimatisation post-importation. However, the data used relates to before 1995 and does not reflect current practices or experience. The one clear fact is that the percentage mortality varies widely between shipments and within shipments. Over the last six to seven years, all imported boxes containing fish have been available for inspection at border inspection posts (BIPs) and there have been no reports of excessive rates of mortality. However, there is no necessity to record mortalities in ornamental fish or to report them. Thus any information available is anecdotal. This surely is an oversight, although one would presume that since the authorities have the power to prosecute over welfare issues, they would do so if mortalities were considered excessive.

There are also few or no published figures for mortality levels of ornamental fish transported within the UK. This is a potential welfare issue in both cases which warrants further research.

The length of the chain of transportation and the methods used in transportation vary considerably. The two extremes of chain might be:

UK producer → Hobbyist
Capture → Holding campsite → Holding facility → Main holding facility → Exporter → Importer → Re-exporter → Importer UK → Wholesaler → Retailer → Hobbyist

With increasing length of chain, there is a potential concomitant decrease in welfare, increase in exposure to pathogens and decrease in accountability. All countries have their own regulations pertaining to transport within their borders: there is a stereotypical view of ornamental fish being transported in clear polythene bags on motorcycles in Asian countries. Within the UK, there would appear to be little enforcement of UK regulations: the Welfare of Animals (Transport) Order 2006 (WATO), although members of the fisheries inspectorate of the Centre for Environment, Fisheries and Aquaculture Science (Cefas) do follow some batches of fish from the airport to the wholesaler in order to monitor unpacking. There would appear to be little or no published research into transport from airport to wholesaler/retailer.

Pre-transport handling

Where fish are farmed or moved initially to a holding facility prior to export, they are generally rested for a few days and treated for any external parasites. It is common practice to starve fish for at least 24 hours prior to shipping to allow gut emptying. Generally, coldwater fish are cooled to a water temperature of 10°C whilst tropical fish are cooled to 20°C. There is no set rate of cooling, although it is recognised that fish are stressed by rapid temperature changes. The above two procedures are aimed at slowing metabolism and decreasing ammonia production during transportation. The density at which fish are packed varies between producers and species and is not specified in the sense of kilograms per cubic metre of water. The packing density to some extent is decided on the producer's experience and economics since it is not profitable to ship water around the world, particularly if the fish die or cannot be sold. Thus there is a commercial imperative to maintain fish welfare to enable profitable sales at full value. However, there would appear to be a difference in the success of transportation between producers and even between farms in the same company. Why this should be so is unclear, but is due to a variety of factors of which the most important is the experience and ability of those working in the packing department. If fish are less than 10 cm in length, several hundred fish can be packed per box but even a slight increase in number can cause the loss of the whole box. Packaging is carried out as close to flight time as possible and usually transport to the aircraft is in temperature-controlled vehicles. The placement in the aircraft hold is

important as – although the pilot can control the hold temperature – if, for example, the boxes are placed too close to the heater outlets then the boxes can overheat.

Air transport

International transport is usually by aircraft and regulated under the International Air Transport Association (IATA) Live Animal Regulations. These regulations are updated yearly and run to several hundred pages. As well as stipulating the minimum requirements for air transportation, they also advise on individual country regulations regarding importation of animals. General requirements for ornamental fish are:

- Fish must be healthy before shipping.
- Most fish, dependent on species, should be placed on reduced rations or starved for several days prior to shipment (the number of days is not stipulated).
- There must be only one species per bag.
- The fish must be packaged in an insulating container.
- The container should be correctly marked and accompanied with the correct importation documents.
- The bag the fish is packed in should contain one-third water and two-thirds oxygen. Whether the fish can swim freely or only freely undulate is dependent on species.
- The container must be leak proof.
- The fish must be packed in such a way that they can survive in the container for up to 48 hours from the time they are accepted by the airline. Airlines are highly unlikely to accept badly packaged fish.
- Care should be taken to reduce heat transfer and exposure to noise to the minimum.

Losses during air transport

Observations made between 1996 and 1998 on the importation of coldwater fish from Israel (C. Walster unpublished data) and more recent anecdotal evidence from the Animal Reception Centre, Heathrow (R. Quest pers. com.), excluding incorrect or failure of the packaging, would indicate three causes of fish losses during international transportation. These are in order of greatest losses:

- Indirect flights
- Delayed flights
- Wrong placement in the hold

These losses are sporadic but can be devastating to an individual shipment with the loss of all fish in a box or group of boxes. What is it that causes these losses? A possible reason for incorrect placement has been discussed above. But why should

an indirect or delayed flight cause losses, particularly when the fish have been packed to survive for 48 hours and the shipment has arrived well within that time? Possible explanations might be:

- Standing on tarmac exposed to the sun, thus causing water temperatures to increase.
- Additional exposure to noise and vibrations due to the extra take off and landing.
- If the boxes are not agitated by movement or vibration then a layer of carbon dioxide can build up at the air/water interface, thus compromising oxygen availability to the fish.
- The build up of toxic waste products in the transportation bags and other deleterious changes in water chemistry.

To try and reduce losses exporters will often add various chemicals to the water prior to shipment. These chemicals include:

- Zeolite and similar to reduce free ammonia (freshwater systems only).
- Phenoxyethanol and other sedatives to decrease metabolism and to reduce aggression in those species that exhibit conspecific aggression, although these fish are usually packaged individually. Use of sedatives should be noted on the IATA label.
- Acriflavine and other antiseptics to reduce potential bacterial build up in the water and on the skin of the fish.
- Salt to reduce osmotic stress (for those freshwater species that are considered salt tolerant).
- pH buffering.

To date, only a few scientific studies have been undertaken to assess the effects of these various additives in terms of decreasing ornamental fish mortality rates during long-distance transportation. However, one study on guppies revealed that a combination of phenoxyethanol (anaesthetic) and either tris-buffer or clinoptilolite (ammonia remover) lowered the mortality rate during air transportation (Teo *et al.*, 1989). Lim *et al.* (2003) discuss more recent improvements in transportation.

In the past, salt has been added to shipments of guppies (*Poecilia reticulata*) in the erroneous belief that they are an estuarine species and that the addition of salt will decrease stress. Even though this error has mostly been corrected, the practice still continues and is also practised with many other tropical species. Calcium chloride has also been used for the same reason for fish from the Amazon (soft water).

There is obviously a need for further research into the air transportation of ornamental fish, but from a veterinary perspective, given the current state of knowledge, it would be reasonable to say that if the following general conditions are met then it is unlikely that fish welfare will suffer:

- Fish should be rested prior to starting the packaging process for at least 48 hours, or longer if an antiparasitic treatment is required.

- Fish should be starved for an appropriate period depending on species prior to transportation.
- Fish should be cooled to the appropriate temperature (10°C for coldwater, 20°C for tropicals) over a suitable period of time, but at least 24 hours.
- The correct number of fish should be packed per box.
- Individual fish appearing to be unwell should not be packed.
- Suitable good quality clean water at the correct temperature should be used for packing.
- Pure oxygen should be used.
- The fish should be packed as close to the departure time as possible.
- Temperature controlled transportation should be used for the journey to the airport.
- A direct flight should be used.
- There should be correct placement in the hold of the aircraft, and the hold temperature should be set correctly.
- There should be no delay in release from the BIP.
- The fish should be picked up promptly by the collector.

All of the above can be achieved through good management and staff training, and it needs to be recognised that this represents best practice within the trade.

Entry into the European Union

For entry into the European Union, all shipments must comply with Directive 91/628/EEC as amended by Directive 95/29/EC, be accompanied by a health certificate valid for the Member State of destination and, where appropriate (for example seahorses, Arowana and corals), comply with the Convention on International Trade in Endangered Species (CITES) requirements. The animals may only be imported direct from a non-EU member state through an approved BIP, which, for tropical aquatic animals entering the UK, are at Heathrow, Manchester, Gatwick and East Midlands airports. The importer must give the State Veterinary Service, Cefas and staff at the BIP at least two working days notice of the number, nature and estimated time of arrival of the animals. In addition, the import must be accompanied by a licence. This licence can be issued by the Fish Health Inspectorate either as an annual licence or for each individual shipment, depending on the country of origin and the species of fish. Where an importer or exporter fails to comply with all of these regulations then there would be, at best, a delay at the BIP or at worst, the shipment would be returned to the country of origin or destroyed. Either eventuality obviously could have an impact on the welfare of the fish. Apart from delays, a welfare issue at BIPs is the opening of boxes to check their contents against documentation. Opening of the boxes and inspection is kept to a minimum and should be carried out in darkened, quiet areas, at an appropriate temperature, and red light is available at the BIP at Heathrow. This is not always the case.

Following the introduction of Commission Decision 2004/319/EC in May of 2004, a new animal health certificate should have accompanied all shipments. However, due to several problems with the new style certificate, not least of which was a failure of the competent authorities in exporting countries to be able to complete it correctly at the beginning of 2005, the old arrangements are still in place. It is envisaged that this certificate should replace the need for a licence.

Transport within the UK

After the fish have passed through the BIP and have been released to the importer in the UK, there can still be a considerable journey time to the final destination. Many wholesalers' premises are within an hour's drive of Heathrow, the main point of entry. Where this journey is extended, the most expeditious method of transport is usually chosen. Due to the availability of flights it is not always possible for the wholesaler to have fish delivered to the most appropriate airport. There are sometimes stories of fish taking up to a further 24–48 hours to reach their destination. Fish may be taken directly to a retailer, to wholesale premises or be distributed through consolidation. Consolidation is a system where smaller retailers may purchase as little as an individual box of fish direct from a producer, by their wholesaler amalgamating several such orders. It is a system that has considerable cost advantages for both the retailer and the wholesaler and potentially the hobbyist. Anecdotal information would indicate that consolidation produces a higher number of claims for fish losses/problems than transport directly to the wholesaler. Again the reasons are unclear, but three possibilities are that journey times are longer, that fish going directly to a retailer are not given time to rest before being put on sale and that there is greater mixing of fish from different origins at a retailer, thus increasing exposure to potential pathogens at a time when the fish's immune system is compromised due to the stress of the journey.

No figures are available for losses occurring during transportation within the UK, but there are areas of concern. Most commercial transportation is carried out in vans with no ability to control temperatures and this can be detrimental to fish on warm sunny days. When this is coupled with delays on motorways with traffic at a standstill then the temperatures within the boxes can rise dramatically. This is particularly important at this stage in the journey since the amount of ammonia in the boxes will be significant, but its toxicity is ameliorated by decreased pH of the transport water due to normal metabolism and, hopefully, still low water temperatures.

Transport losses

Accurate figures for ornamental fish losses due to transportation are hard to quantify, although this would be a fairly easy welfare issue to research. The scientific literature indicates that it can be very high (Huntingford *et al*. 2006), and this would

not be disputed for individual shipments. It needs to be recognised that transportation around the world of a number of ornamental species is a relatively recent phenomenon, and this could explain such losses, but it might be argued that 30+ years should be enough time to have got things right. However, the statistics of losses from even a few years ago should be treated with caution. Like many areas of aquaculture the knowledge and ability to keep, and successfully transport species has increased dramatically within the last decade.

Defining transportation losses as any deaths that occur prior to reception at the place of destination, then anecdotal information might indicate that on average, over the course of a year, between 0.5 and 1.5% of ornamental fish imported into this country die due to transportation. This average figure is clearly influenced markedly by the condition of the fish prior to shipment, such as body score and health status, but one of the biggest influences is mistakes in packaging, which can be summed up as human error such as over-packing, packing the wrong tank of fish, using the wrong water or 'oxygen'. If one were to include losses occurring immediately after transportation (1–2 weeks) then this figure would easily double. But it should be remembered that these losses are often due to poor reception or packing practices.

These figures do not compare favourably with transportation losses in other farmed species, and in particular those for the transportation of other young animals such as lambs or chicks which, although their journey times would be significantly less, are in the region of 0.25 to 1%. Consequently, from a veterinary perspective, the losses seen in ornamental fish during transport are unacceptable. However, the transportation of ornamental fish is not as regulated (at least in terms of specified requirements), or at the very least the regulations are not enforced as rigorously as for other animals; also our knowledge of the requirements and effects of transportation on ornamental fish is far from complete, and more research is needed on this subject.

Arrival at destination

Wholesaler premises

Reception protocols at the final destination vary significantly between companies but have a significant impact on the eventual total numbers of losses due to transportation. These protocols differ depending on facilities available, the knowledge of personnel, and practices carried out. It is clear that the reception protocols used and the management ability of the receiving wholesaler are paramount in affecting the number of mortalities at this point and also the speed with which the fish recover from the stress of the journey (C. Walster unpublished data). As examples, some companies will dip fish in salt or potassium permanganate solutions upon arrival, netting the fish out of the dip and placing into the holding facility. Weak fish will succumb to this procedure and they, along with any other dead fish, can be removed immediately. The procedure has the advantage of being quick and removing all fish

likely to fade over the next few days from the stress of transportation. In terms of economics it is probably advantageous but certainly does not have the welfare of fish at its heart. Other companies will carry out quite complex procedures taking several hours to complete. The aim of these procedures is to decrease the effects of transportation by allowing ammonia built up in the transportation water and the fish to be flushed to waste whilst minimising any increase in ammonia toxicity due to increased temperature and pH. This method can be labour and time consuming but obviously has the welfare of the fish as the prime motive. There is clear evidence that carrying out a reception procedure along these lines markedly decreases any problems with transportation.

OATA guidelines recommend that all fish should be kept isolated for at least 48 hours after arrival in a quiet darkened area. During this period, food should only be given sparingly and an assessment made of any remedial treatments required.

Minor problems connected with transportation include bruising and split fins, often due to rough handling, and ammonia burn where the extremities of the fish become necrotic due to ammonia toxicity, probably relating to poor packaging procedures at the place of export. These problems occur sporadically and it would be difficult to prevent them completely. Of major concern is the loss of fish during transportation and losses occurring over the following week or two, due to fish succumbing to secondary infections. These losses occur for a variety of reasons as outlined above and can be significant for individual shipments. Where losses are significant for an individual shipment then it is likely to be due to a combination of factors rather than one individual reason and is more likely to occur when the fish are in poor condition prior to transportation or be due to poor packaging.

Hobbyist premises

The knowledge of hobbyists is variable and, as such, several problems can arise once the fish arrive at their final destination. New hobbyists might purchase fish which are unsuitable for their aquarium set up, for example by mixing fish with different hardness requirements, choosing fish which will grow too large for the tank or are unsuitable as community fish. Failure to provide sufficient time to match water temperature with the aquarium or pond prior to release can also occur. Such things can happen despite the fact that best practice dictates that retailers should provide appropriate information at the point of sale. Provision of such information is a legal requirement of the Animal Welfare Bill.

Fish health

Like other areas of aquaculture, there are concerns over emerging diseases, the biosecurity of facilities and the ability to treat disease due to a lack of pharmaceuticals. For example, there are no vaccines available for use in ornamental fish and

even where licensed products are available they are packaged in quantities more applicable to fish farm use than for an aquarium. Many fish remedies are available over the counter, and make fairly wide ranging claims as to efficacy that from a practical perspective they do not appear to live up to. Since veterinary surgeons tend to be the last place of call for hobbyists experiencing fish health problems, it is often the case that the fish have been exposed to a cocktail of chemicals when seen, which can cause more problems than the initial complaint. This though is not entirely the fault of the trade, as hobbyists will often seek advice on the same problem from several sources, and this advice may well vary.

There are two major welfare concerns connected to fish health. The first are the various health issues affecting fancy goldfish, ranging from fin damage in long-finned varieties, impaired vision in bubble eyes and orandas due to excess skin, and swim bladder problems in the majority of fancy varieties, notably the round-bodied forms.

The second is the lack of involvement of the veterinary profession in ornamental fish health. For over 100 years, fancy goldfish have provoked arguments between those who see them as man-made freaks and those who feel they are a connection to the culture of ancient China. It is unlikely that this argument will ever be resolved. From a veterinary perspective, this group of fish is prone to a number of conditions, the most significant of which are chronic problems of the swim bladder, since this can ultimately prove fatal to the fish. As with many things to do with ornamental fish, there are no statistics as to the percentage of fancy goldfish which are affected by swim bladder problems, or by how much the average life is shortened, but swim bladder problems are one of the top reasons a hobbyist will contact a veterinary surgeon, or helplines run by manufacturers of aquarium and pond equipment, an example of which would be the Aquarian Advisory Service, and they are almost invariably related to varieties of fancy goldfish. Unless the problem is dietary in origin and can be resolved by changes in management then it is likely that despite treatment, the animal's life will be shortened. It is often difficult to diagnose the exact problem in the live animal. Even though changes in size, shape, thickness and contents can be visualised through a variety of techniques, it is likely that the rounded body shape of certain fancy goldfish varieties has contributed to the problem. Interestingly, one could sum up the breed standards of these species as fish exhibiting 'harmonious balance' which might indicate that top class examples do not suffer from swim bladder problems to the same extent as their more widely available compatriots. Treatment ranges from the use of salt to antibiotics and even surgery, but often only has a short-term benefit. Surgical treatments, which range from the attachment of external flotation devices, deflation by aspiration, to the insertion of small pieces of 'gravel' have never been assessed properly, and give rise to further welfare concerns. As with other companion animals, it is clear that the requirements of the breed standard have compromised the health of the animal and it is worth noting that in the past few years the Kennel Club has started to revise standards for those canine breeds where previous standards have clearly

compromised health and welfare. Perhaps the goldfish societies should follow suit.

The lack of involvement of veterinary surgeons in ornamental fish health is mainly due to historical reasons, but has had a number of consequences all of which are to the detriment of the fish. Some of these are speculative, such as the failure of the pharmaceutical companies to become involved in ornamental fish, or a failure of the standard of health care to keep pace with improvements seen in the care of other companion animals. Some are clear examples, such as where the failure of the profession has led to untrained people carrying out surgery. The type of surgery concerned ranges from clipping split fins to full internal abdominal surgery. What is even more disturbing is that information on how to carry out these procedures has been published in hobbyist books and magazines, and even web sites advertise 'surgical' kits. For example, hobbyist magazines and books have published articles on: removal of a skin tumour using laser therapy (*Koi Carp Magazine*, March 2003); removal of abdominal fat and the ovaries, the weight of which was felt to be causing an imbalance which prevented the fish from swimming correctly (*Koi Carp Magazine*, February 2003); and insertion of gravel into the abdominal cavity to correct imbalance due to swim bladder disorders (Johnson & Hess 2001) This last article states: 'And finally, erring on the side of using less anaesthetic and seeing a little squirming is better than killing the fish with excessive anaesthesia'. Surely it would be better to advise the hobbyist that if they are not capable of carrying out a safe anaesthetic then they should not try?

This situation is to be lamented and the veterinary profession should accept its share of the blame. There are arguments for and against the provision of medical treatments by veterinary surgeons or those with suitable experience in the trade or hobby. The veterinary argument might be that there would be better diagnosis and less use of inappropriate antibiotics that have been illegally sourced. The trade argument might be that their costs are cheaper and the service is better and more widely available since less than 10% of veterinary practices have expressed an interest in treating ornamental fish. However, the main concern is the fact that surgery is being carried out by untrained people and that under current legislation there is little that can be done to stop it.

General considerations

Given that ornamental fish are considered as companion animals, then there is an obligation on the keeper to provide for the appropriate welfare. Does the industry and hobby provide that level of welfare in the UK?

It is only within the past 50 years or so that the keeping of fish has become more popular and the keeping of more exotic species has increased. This has occurred due to the ease and speed with which fish can be transported around the world, coupled with improvements in aquarium equipment, easier maintenance of

systems and the increasing abilities of the hobbyist. During this same period, public concerns regarding the welfare of all animal species has increased along with an awareness of the impact human activity has on the environment and the animals that live within that environment. These concerns have led to questions being raised over the sustainability of the industry and the suitability of some species for ornamental purposes. Both hobbyists and the trade in particular have responded actively and positively to these concerns. The Ornamental Aquatic Trade Association (OATA) published their guidelines on the minimum water quality requirements for ornamental fish in 1993 and have provided distance learning materials since 1998. There are a number of colleges such as Sparsholt and Brooksby providing training courses in ornamental fish management up to BSc standard, and Plymouth University provides an Aquarium Science and Conservation module at postgraduate level, the module being part of the MRes degree in Applied Fish Biology. For the hobby, there is a wealth of information available in magazines, books, local clubs and on the internet, but there is a problem of variability in quality of information. If the provision of good welfare were to be based on the availability of sound information on how to provide such things as the correct environment, nutrition and suitability of species then the answer to whether or not sufficient care is taken over ornamental fish welfare would be a definite yes.

Problems arise though due to misidentification of fish species by the trade, incorrect information being handed out to hobbyists or correct information being ignored. This leads to the three major welfare factors associated with the keeping of ornamental fish in the United Kingdom:

- Lack of adequate housing space. A small volume of water is universally recognised as being difficult to manage. OATA recommendations are that fish are kept in an absolute minimum of 4 litres of water and that beginners should think of nothing less than 10 litres. Recently, a number of mini-aquariums have been on sale with a water volume of less than 1 litre, none of which are supported by trade bodies.
- Unsuitable selection of species for mixed species (community) aquariums can result in social or environmental (notably chemical) incompatibility problems.
- Lack of understanding of the environmental needs of fish, and the associated life support systems (e.g. the principles of biofiltration) necessary to satisfy these needs in captivity, can lead to welfare problems.

There is a large body of legislation which has an impact on the trade and hobby within the UK, much of which is in the process of being updated, and this should help to ensure the minimum welfare standards that society expects. There are, however, variations across the country on how strictly this legislation is applied, for example with the criteria necessary to obtain a pet shop licence or hold a livestock show. Hobbyists are generally ignorant of this legislation, particularly with regard to medicines and the release of non-indigenous species.

From a trade perspective, OATA has long played an active role at national level in forming relevant standards to be applied to pet shop licensing. However, this

licensing is carried out by environmental health officers who often ignore simple inexpensive water quality tests to monitor husbandry in aquatic outlets.

No fish-keeper has ever been successfully prosecuted for cruelty and bodies such as the RSPCA seem to demonstrate little detailed knowledge of fish. This apparent lack of interest in fish welfare has been exemplified by the Government U-turn on their original pledge to ban goldfish as prizes at fairs. Some would argue that codes of conduct introduced under secondary legislation have effectively negated this apparent U-turn, but surely this gives out the wrong message to the fish-keeping (and angling) communities. All of this is perhaps a reflection on public perception of fish and fish welfare.

Conclusions

For an ornamental fish in the UK there are potentially four ‘life stages’. These are: production or collection, transportation, UK trade and finally UK hobby. There are apparent welfare issues at all four stages but each stage needs to be considered separately due to the different issues involved and potential resolutions. For all four stages there is a lack of scientific data that restricts debate on issues arising and thus the debate often hinges on observations or anecdote rather than fact. It should be pointed out that for a fish to be traded as an ornamental it must be commercially viable (although it is acknowledged that some species, especially certain wild-caught marines, are simply not robust enough to survive the stresses of capture and transportation, yet attempts are still made to trade them). This means that it must be able to withstand the rigours of transportation and be kept alive by the hobbyist. Therefore prior to trading a species it is necessary to know the:

- Water quality and basic environmental requirements.
- Nutritional requirements and availability of appropriate food.
- Suitability of keeping the species in a tank or pond environment.

By default this means that basic welfare requirements are established prior to trading. Then, through the interaction between the various national and international trade organisations, relevant government bodies and hobbyist groups, there is a continuous feedback to producers as to the suitability of their fish for ornamental purposes and any issues of concern can be raised. Thus the UK hobbyist and trade do, and continue to, influence the conditions in which fish are produced and transported, and it should be recognised that this is a dynamic, ongoing process. Within the past decade, this has led to significant improvements in production and transportation welfare, and the trade bodies such as OATA have been instrumental in this success. Best practice in the industry, although not widely reviewed by appropriate professionals, can be seen to be very good, or excellent in many instances.

Within the UK there are many issues which raise welfare concerns both for the trade and hobby. These issues are as diverse as reception practices for fish at retailers, exhibiting fish at shows, transportation in the UK, the ability of new

hobbyists to keep fish and the ability of more experienced hobbyists in treating fish. From a veterinary perspective, failures in these areas indicate poor welfare but it would be very unfair to paint a completely black picture. There simply is no proper data on what actually is happening to fish once they arrive in the UK, and the majority of vets working in this area would recognise that their experiences are probably bleaker than most. One thing that would seem clear is that these issues could be resolved relatively easily and this will be discussed below.

How does the welfare of ornamental fish in the UK compare to that of other companion animals? Surprisingly, from a veterinary perspective, the answer may well be quite favourable when set against such known criteria as the numbers of stray dogs, feral cats, the number of RSPCA prosecutions for cruelty, the percentage of companion animals seen by the veterinary profession each year, delays in seeking treatment by owners and even failure to seek treatment at all. There are estimated to be 20 times as many ornamental fish in the UK as there are pet dogs and cats and, as a consequence, it seems unlikely, even with the paucity of statistics available, that the number of welfare incidences in ornamental fish will be significantly higher in percentage terms than in dogs and cats. However, it could also be argued that the paucity of information hides the magnitude of the problem. For example, unwanted fish are often flushed down the toilet or killed by a blow to the head, so comparisons with unwanted cats/dogs cannot be made. Also, the RSPCA openly admit to the public that they are not really interested in fish. So there is likely to be a gross under-reporting of fish welfare issues.

Further research into the needs of ornamental fish is clearly necessary, and a mechanism for funding needs to be found. The trade itself already contributes and is involved in Defra co-funded Link Aquaculture projects, but it is unlikely that the trade could be persuaded to contribute more, or that it could afford to do so.

Better education for the trade and hobbyists is also necessary. OATA is already active on this front through its codes of practice and certificate and diploma courses aimed at the trade. Recently, in conjunction with one of the hobbyist magazines, the material used in these courses has become available to the hobbyist. OATA has already recognised that hobbyists should receive correct information on the care of fish at the point of sale and the Animal Welfare Bill also recognises this need. OATA represents around a third of retail outlets by number, and a far greater percentage by turnover, but the entire trade needs to be included in this matter.

Through the availability of books, magazines, the internet and local clubs, the hobbyist has a wide range of information at their disposal. However, some of the information is incorrect and the hobbyist may well not be able to identify which this is. This problem occurs mainly in printed material and on the internet. For the new hobbyist this can be a major problem compounding the fact that they have often not researched the necessary information to be successful in their new hobby. Initial purchases of fish are often done for aesthetic rather than practical reasons and regularly, newly bought fish will die of 'new pond/tank syndrome' resulting in the hobbyist losing interest in ornamental fish altogether. Those that

get past this stumbling block often find an appetite for becoming better informed and often acquire greater knowledge than some 'experts'. This suggests that there should be a requirement for a new hobbyist to have passed an 'exam' in basic fish husbandry, and/or a mechanism for providing a licence to keep ornamental fish. This might be justified on the basis that the aquatic environment is relatively alien to humans, compared to the terrestrial environment of most other pets such as cats and dogs. Although this might solve some welfare issues it is unlikely that it would receive widespread support and if it were implemented there would be inevitable administrative problems.

Greater involvement of the veterinary profession should be encouraged. At present the veterinary colleges spend little time on fish. This comes about due to a crowded timetable and no statutory requirement to provide education in this field. Inclusion of fish in a new Veterinary Surgeons Act would provide the statutory requirement, but equally there needs to be a market for these services which at present does not exist. However, for the demand to develop, the veterinary profession must first ensure that active practitioners are equipped with the skills necessary to provide a creditable service. The creation of this market depends on many factors of which a key one is that the public perception of fish needs to change. Currently they are often seen from an economic perspective, that is, it is cheaper to buy a new one than pay for treatment even when treatment is available; or that they are simply not worth the effort. These days, some fish cost the same as a bar of chocolate and as a result they are often treated as a disposable item. Fish need to achieve the same status as other companion animals whereby veterinary intervention is sought on emotional rather than economic grounds.

The way forward can be summed up as:

- Greater availability of information to the hobby.
- Better training and understanding to ensure all commercial enterprises are adequately equipped to provide good husbandry and health care.
- Vets receiving the appropriate training to ensure the availability of good health care and husbandry advice to both the trade and hobby.
- Ensuring that the standard guidelines under the Pet Animal Act 1951, and/or any codes of practice developed under the Animal Welfare Bill, are evenly and effectively applied.
- Fish receiving the same protections as other vertebrates.

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